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DEVELOPMENT OF METHOD FOR SEMIVOLATILE ORGANIC PRIORITY POLLUTANTS IN FISH

by

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A method was developed for the determination of neutral and acidic priority pollutant compounds in fish tissue. Priority pollutant compounds are extracted from the fish tissue during homogenization of the tissue in acetonitrile. The acetonitrile extract is isolated from the tissue after centrifuging and placed in a tumbling bottle containing salted water buffered to pH 4, methyl t-butyl ether (MTBE), and petroleum ether. The mixture is equilibrated by tumbling and a portion of the organic layer is isolated after drying over sodium sulfate. The acetonitrile partitioning step serves to remove a portion of the extractable fish lipid material from the priority pollutant compounds. The organic extract is concentrated and the remaining interfering lipid material is removed using a gel permeation chromatography (GPC) procedure. The final extract is concentrated and priority pollutant compounds are detected and quantified in the extract by gas chromatography-mass spectrometry (GC-MS). The method yields a concentration factor of 10 and detection limits for the neutral and acidic priority pollutant compounds in fish tissue in the mid to high parts per billion (w/w) range.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory-Cincinnati conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.

- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report summarizes the development and validation of a method to determine semivolatile priority pollutant compounds in fish tissue.

Robert L. Booth, Director
Environmental Monitoring and Support
Laboratory-Cincinnati

ABSTRACT

A method was developed for the determination of neutral and acidic priority pollutant compounds in fish tissue. Priority pollutant compounds are extracted from the fish tissue during homogenization of the tissue in acetonitrile. The acetonitrile extract is isolated from the tissue after centrifuging and placed in a tumbling bottle containing salted water buffered to pH 4, methyl t-butyl ether (MTBE), and petroleum ether. The mixture is equilibrated by tumbling and a portion of the organic layer is isolated after drying over sodium sulfate. The acetonitrile partitioning step serves to remove a portion of the extractable fish lipid material from the priority pollutant compounds. The organic extract is concentrated and the remaining interfering lipid material is removed using a gel permeation chromatography (GPC) procedure. The final extract is concentrated and priority pollutant compounds are detected and quantified in the extract by gas chromatography-mass spectrometry (GC-MS). The method yields a concentration factor of 10 and detection limits for the neutral and acidic priority pollutant compounds in fish tissue in the mid to high parts per billion (w/w) range.

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SECTION 1

INTRODUCTION

The purpose of this work assignment was to develop, evaluate, and validate a gas chromatography-mass spectrometry (GC-MS) method to determine semivolatile organic priority pollutant compounds in fish. The extraction and cleanup procedures used in the final method were based on the method entitled "Analysis of Fish for General Organics by Solvent Extraction" presented in the EPA publication entitled "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue" (EPA 600/4-81-055). Analysis procedures used in the final method were based on EPA Method 625 which specifies the use of GC-MS.

Prior to initiation of the work assignment, the EPA Project Officer conducted a phone survey of laboratories active in the area of fish analysis. Laboratories contacted were requested to supply information and methods related to the determination of organic compounds in fish tissue. The EPA Project Officer provided the results of the phone survey as a basis for development of a draft analysis method.

A computerized literature search using the Chemical Abstracts Service (CAS) On-line system was conducted to obtain additional information concerning the determination of semivolatile organic priority pollutant compounds in fish. References were identified by searching the CAS On-line data base for the CAS registry numbers of each of the semivolatile organic priority pollutant compounds. The search was limited to literature discussing the analysis or determination of the semivolatile organic priority pollutant compounds in fish and tissue, published in English in journals after 1966.

Information obtained by the EPA Project Officer from the phone survey of laboratories active in the area of fish analysis is summarized in Table 1. A summary of the information obtained from the computerized CAS On-line literature search is presented in Table 2.

Information obtained from the literature survey was summarized into three categories: homogenization techniques, extraction techniques, and cleanup techniques. The techniques, as well as some advantages and disadvantages associated with each technique, are presented in Tables 3-5.

TABLE 1. SUMMARY OF FISH ANALYSIS INFORMATION OBTAINED BY EPA TASK OFFICER DURING PHONE SURVEY

SOURCE	EXTRACTION PROCEDURE	CLEANUP PROCEDURE	COMMENTS
EPA Interim Methods for Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue	Homogenization in CH_3CN; partition into hexane	GPC if required	
Basic Water Monitoring Program Guidelines for Fish Analysis	Na_2SO_4; column extraction with ethyl acetate/ petroleum ether	GPC; silica gel	Pesticide method
	Soxhlet extraction with acetone/hexane	GPC	Pesticide method
Rogerson, Narragansett RI(verbal)	Blender extraction with acetone/Freon 113	Silica gel	Pesticide method

TABLE 2. SUMMARY OF FISH ANALYSIS INFORMATION OBTAINED FROM COMPUTERIZED LITERATURE SEARCH

	REFERENCE	COMPOUND	MATRIX	FOUND, PPB	LIMIT, PPB	EXTRACTION METHOD	CLEANUP METHOD	ANALYSIS METHOD
1	Anal. Chem., 51(7), 989-92 [1969]	N-Nitrosodimethylamine	Fish	.035-.25	.001	Steam distillation	None	Packed GC-MS
2	Anal. Chem., 50(1), 182-5 [1978]	Pesticides/PCBs/phenols/PAHs	Fish	Detected	Not reported	Na2SO4/Soxhlet	GPC	Packed GC-MS
3	J. Agric. Food Chem., 25(3), 680-2 [1977]	Hexachlorobenzene/pesticides	Rat tissue	Not reported	Not reported	Tumbling with CH3CN/hexane	None	Packed GC-ECD
4	J. Agric. Food Chem., 17(4), 871-3 [1970]	Pentachlorophenol	Fish	100,000	Not reported	Base hydrol/steam distil/tumbling/deriv	None	Packed GC-ECD/MS
5	J. Ass. Off. Anal. Chem., 64(2), 282-6 [1981]	Bis(2-ethylhexyl)phthalate	Fish	1-10,000	1	Homo in acetone/hexane	GPC/alumina	Packed GC-ECD
6	J. Ass. Off. Anal. Chem., 63(1), 27-32 [1980]	Chlorophenols/chlorobenzenes	Fish/other tissues	Not reported	Not reported	Acid dig/extr benzene/hexane	Silica gel/methylate/alumina	Packed GC-ECD
7	J. Ass. Off. Anal. Chem., 59(3), 559-61 [1976]	Hexachlorobutadiene	Fish/milk	10-1200	1	Not reported	Florisil	Packed GC-ECD
8	J. Ass. Off. Anal. Chem., 59(1), 174-87 [1976]	Hexachlorobenzene/pesticides/PCBs	Animal fat	Not reported	Not reported	Not reported	GPC	Packed GC-ECD
9	J. Ass. Off. Anal. Chem., 58(3), 557-61 [1975]	Hexachlorobenzene	Fats and oils	Not reported	Not reported	Not reported	Florisil	Packed GC-ECD
10	J. Ass. Off. Anal. Chem., 58(1), 1-5 [1975]	Pesticides	Fish	Not reported	1-10	Na2SO4/Soxhlet	Micro Cel-E/GPC	Not reported
11	J. Ass. Off. Anal. Chem., 57(3), 576-9 [1974]	Pesticides/PCBs	Fish	10-2000	10	NaSO4/column elution	Florisil/silica gel	Packed GC-ECD
12	J. Ass. Off. Anal. Chem., 55(6), 1360-2 [1972]	Pesticides/PCBs	Animal tissue	Not reported	Not reported	Not reported	Acid digestion	Not reported
13	J. Ass. Off. Anal. Chem., 55(1), 32-38 [1972]	Pesticides/PCBs/phthalates	Fish	Not reported	Not reported	Dry ice homo/blender,column extr	GPC	Packed GC-ECD
14	J. Ass. Off. Anal. Chem., 54(3), 499-506 [1971]	Pesticides	Fish	Not reported	Not reported	NaSO4/Pet ether extr	CH3CN part/Florisil/silica gel	Packed GC-ECD
15	Bull. Environ. Contam. Toxicol., 7(2/3), 115-20 [1972]	Pesticides/PCBs	Fish	33-860	Not reported	Dry ice homo/NaSO4/column extr	CH3CN part/GPC	Packed GC-MS
16	Bull. Environ. Contam. Toxicol., 25, 305-312 [1980]	Pesticides	Fish/shellfish	0.1-15	Not reported	NaSO4/hexane extr/CH3CN part	Florisil	Packed GC-ECD
17	J. Ass. Off. Anal. Chem., 50(3), 646-50 [1967]	Di-n-octylphthalate	Milk lipids	Not reported	Not reported	Not reported	Alumina	Packed GC-ECD
18	J. Chromatogr., 111(2), 426-429 [1975]	N-Nitrosodimethylamine	Fish meal	Not reported	Not reported	Vacuum distillation	None	Not reported
19	J. Chromatogr., 276(1), 77-84 [1983]	2,4-Dinitrophenol	Tissue	Not reported	100-200	Homo/extr ethyl ether	Basic backextr	Packed GC-AFD/MS
20	Bull. Environ. Contam. Toxicol., 24, 257-64 [1980]	Phenols	Tissue	10-100	Not reported	Acidification/extr ether	Basic extr with ether	Cap GC-MS
21	Environ. Sci. Technol., 13(3), 329-33 [1979]	3,3'-Dichlorobenzidine	Fish	Not found	100-5000	Basic dig/extr benzene	Acid wash/GPC	Packed GC-ECD
22	Bull. Environ. Contam. Toxicol, 25, 59-64 [1980]	Pesticides/PCBs	Tissue	Not reported	1-20	Hexane extr	GPC/CH3CN part/Florisil	Packed GC-NPD
23	Bull. Environ. Contam. Tox., 17(6), 631-6 [1977]	Pesticides/PCBs	Tissue	Not reported	Not reported	Steam distillation	None	Packed GC-ECD
24	Bull. Environ. Contam. Tox., 12(3), 301-7 [1974]	Pesticides/PCBs	Fish	20-100	Not reported	NaSO4/column,Soxhlet extr	None	Not reported
25	J. Ass. Off. Anal. Chem., 50(3), 605-15 [1967]	Pesticides	Oils	Not reported	Not reported	Sweep co-distillation	Florisil	Packed GC-ECD
26	J. Ass. Off. Anal. Chem., 50(3), 581-5 [1967]	Pesticides	Milk	Not reported	10	Extr methanol/ethyl acetate	Sweep co-distillation	Packed GC-ECD
27	Environ. Sci. Technol., 16, 310-318 [1982]	Toxaphene	Fish	Not reported	1	Na2SO4; column extr	GPC	Packed GC-TID
								Cap GC-ECD/MS

TABLE 3. OPTIONS FOR FISH TISSUE HOMOGENIZATION

PROCEDURE	ADVANTAGES	DISADVANTAGES
1. Blender	More repeatable homogenization; durable equipment	Possibility of sample contamination
2. Tekmar Tissumizer or Polytron	Sample contamination unlikely; yields better particle size reduction than other procedures	Equipment problems may arise if anhydrous salts are present
3. Homogenize in presence of Dry Ice	Aids in grinding of tough samples	Time-consuming; probably unnecessary procedure; Dry Ice may be a source of contamination

TABLE 4. OPTIONS FOR FISH TISSUE EXTRACTION

PROCEDURE	ADVANTAGES	DISADVANTAGES
1. Homogenize buffered fish with organic solvent; tumble; centrifuge	Simple; direct; proven effective with fatty samples	Possible problems with emulsion formation
2. Grind fish in mortar and pestle with Na_2SO_4 to dry; extract in Soxhlet	Optimum extraction; no emulsion formation; no centrifugation or filtration step necessary	Use of Na_2SO_4 might lead to formation of clumps which are not well extracted; no pH control
3. Homogenize fish with Na_2SO_4 and solvent; extract in Soxhlet	Optimum extraction; no emulsion formation; no centrifugation or filtration step necessary; no clumps formed	No pH control
4. Grind fish in mortar and pestle with Na_2SO_4 to dry; extract in column	No emulsion formation; no centrifugation or filtration step necessary	Use of Na_2SO_4 might lead to formation of clumps which are not well extracted; no pH control; probable inefficient extraction
5. Homogenize fish with Na_2SO_4 and solvent; extract in column	No emulsion formation; no centrifugation or filtration step necessary; no clumps formed method	No pH control; probable inefficient extraction
6. Steam distillation	Lipids removed; no cleanup necessary	Poor recoveries for high-boiling compounds, e.g. dibenzo(a,h)anthracene

TABLE 5. OPTIONS FOR FISH TISSUE EXTRACT CLEANUP

PROCEDURE	ADVANTAGES	DISADVANTAGES
1. Adsorption chromatography (Florisil, silica gel, alumina...)	Easy; inexpensive; requires no automation	Not effective for removing large amounts of lipids from most priority pollutants
2. Acetonitrile partitioning	Easy; inexpensive; requires no automation	Compound recoveries vary by their distribution coefficients; may not be effective for recovering phenol and nitrophenols; difficult to remove CH_3CN from extract
3. Sweep co-distillation	Looks promising; literature indicates effective removal of lipids	Technique not routinely used; must be automated to be considered; may not be effective for recovering high-boiling compounds, e.g. dibenzo(a,h)anthracene
4. Gel permeation chromatography	Reusable resin column; automation possible; effectively removes more than 99% of lipids from extract	May not effectively isolate phthalates from lipids

Results of the literature survey were presented to the EPA Task and Project Officers and the following guidelines were formulated for development of the fish analysis method:

(1) The fish analysis method would be based on the method entitled "Analysis of Fish for General Organics by Solvent Extraction" presented in the EPA publications entitled "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue" (EPA 600/4-81-055). The Interim Method specifies homogenization of the fish in acetonitrile, partitioning the acetonitrile extract between water and hexane using a separatory funnel, and optional use of GPC to remove excess fish lipid material.

(2) Because of the resource limitations no attempts would be made to ruggedize the acetonitrile extraction procedure.

(3) Cleanup procedures would be limited to the use of acetonitrile partitioning and/or GPC.

(4) The determinative step would be GC/MS Method 625 with a capillary GC column.

(5) A group of compounds considered to be representative of the semivolatile organic priority pollutants would be chosen for use during the preliminary laboratory experimentation phase of the work assignment. The work assignment was divided into two phases. The first phase of the work assignment consisted of preliminary laboratory experimentation as required to establish promising fish extraction and cleanup techniques. The first phase of the work assignment included instrumental range studies and preliminary evaluation of the draft method. The second phase of the work assignment included method range and detection limit studies and matrix validation studies.

(6) Extraordinary efforts would not be made to include the two basic compounds listed in EPA Method 625, benzidine and 3,3-dichlorobenzidine, because of instability problems.

(7) The method would be designed to use a fish sample size of approximately 10 to 25 grams.

(8) If possible, the method would be designed such that it could be easily expanded to include the determination of other semivolatile organic compounds in fish.

Method validation work was conducted in accordance with the "Single Laboratory Validation Protocol." The draft method developed is included as part of this report as Appendix A.

SECTION 2

CONCLUSIONS

An effective analysis method was developed for the determination of neutral and acidic priority pollutant compounds in fish. The following conclusions were made during the Work Assignment:

- Historical information regarding the determination of priority pollutant compounds in fish tissue is sparse. Most available information references the determination of nonpolar pesticides in fish samples.
- The acetonitrile partitioning procedure is effective in removing approximately three-fourths of extractable fish lipid material. Composition of the organic phase used during the acetonitrile partitioning procedure does not have a significant effect on lipid removal.
- Use of polar solvents such as MTBE, as opposed to nonpolar solvents such as hexane, results in significantly higher recoveries of polar priority pollutant compounds (phenols).
- Use of a single tumbling extraction technique as opposed to triplicate extractions using a separatory funnel results in equivalent or superior recoveries of priority pollutant compounds. The tumbling extraction technique is more easily controlled than separatory funnel techniques, resulting in a more rugged and less operator-dependent analysis procedure.
- GPC effectively removes in excess of 98 percent of fish lipid material from the sample extract. However, initiation of GPC fraction collection may have to be adjusted on a matrix-to-matrix basis depending upon the lipid composition of the fish sample. Delaying collection of the GPC fraction may decrease recoveries of long-chain phthalates.
- Instrument range studies indicate that the method will allow detection limits of most of the priority pollutant compounds in the mid to high ng/g level in fish.

- Recoveries of priority pollutant compounds spiked into fish in the mid ng/g to the low µg/g concentration levels are generally greater than 50 percent. Storing the sample for 24-hours prior to processing results in slightly lower but not statistically significantly lower recoveries of most of the compounds.
- Although instrument range studies indicate that detection limits in the range of 20 ng/g can be achieved, demonstrated method detection limits were closer to 200 ng/g for most of the priority pollutant compounds.

SECTION 3

RECOMMENDATIONS

Based on the studies conducted in this work assignment, the following recommendations are made:

- Additional studies are desireable to determine how the characteristics of differing fish matrices will change analysis results. The matrix studies conducted in this work assignment indicated that matrix effects may be a significant issue.
- The method is designed to be rugged, although studies may be advisable to determine how slight changes in method performance might affect analysis results. Areas that might be studied include GPC and MS instrumentation parameters.
- The method may also be applicable to other compounds of environmental interest such as additional targeted pesticides.

SECTION 4

METHODOLOGY

PRELIMINARY LABORATORY EXPERIMENTATION

An acetonitrile extraction and partitioning procedure is outlined in the EPA "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue." Organic materials are extracted from the fish tissue by homogenizing the wet tissue in the presence of acetonitrile. Lipid material is then removed from the extract by partitioning in a mixture of acetonitrile, salted water, and hexane. The EPA Interim Method specifies that 20 grams of fish be extracted with acetonitrile by homogenizing the tissue three times with 60 mL each of acetonitrile. The acetonitrile extracts are combined and added to 1300 mL of 2 percent sodium sulfate in a 2-liter separatory funnel. The pH of the aqueous solution is adjusted first to pH>10 and then to pH<2. The aqueous solution is extracted three times with 60 mL each of hexane at each pH value. Although the EPA Interim Method is specified for use in the determination of all of the semivolatile organic priority pollutants, no validation data are presented. Recoveries of polar compounds such as the phenols into the hexane were not expected to be satisfactory using this procedure. The EPA Interim Method requires multiple extractions to be performed, making performance of the method tedious, slow, and subject to operator-incurred variability. Preliminary laboratory experimentation studies were designed to use the EPA Interim Method as a basis for development of an improved fish analysis method.

Acetonitrile Partitioning Studies

Studies were conducted to determine if the acetonitrile partitioning procedure could be expected to removed lipid material from the fish extracts without significant recovery losses of the priority pollutant compounds. Initially, studies were conducted in the absence of fish lipid material to eliminate matrix effects. Selected priority pollutant compounds, 5 mg each, were added to a 1-liter bottle containing 75 mL of acetonitrile, 500 mL of

distilled water containing 2 percent sodium sulfate and 1.5 percent monobasic potassium phosphate (pH 4.0), and 150 mL of a selected organic solvent or organic solvent mixture. Organic solvents investigated included combinations of hexane, petroleum ether, methylene chloride, and MTBE. Each equilibration was done in triplicate. Individual priority pollutant compounds expected to represent analytical difficulties were selected for the study. The bottle was capped, placed in a tumbler, tumbled end-over-end for one hour, and allowed to sit for one hour. The contents of the bottle were poured into a 2-liter graduated cylinder and the volumes of the aqueous and organic phases were measured. The organic phase was removed for analysis by GC-FID to determine the percent recovery of each of priority pollutant compounds. The organic phase was also analyzed by GC-FID to determine acetonitrile content. The EPA Interim Method specifies that the aqueous phase be adjusted to pH<2 prior to extraction. Recoveries of priority pollutant compounds into hexane were also studied at pH 1.5 for comparison to the above mentioned data obtained when the aqueous layer was adjusted to pH 4.

Acetonitrile Partitioning Cleanup Efficiency Studies

The mechanism by which the acetonitrile partitioning procedure works is not obvious. Presumably, the majority of the acetonitrile and the lipid material remain in the aqueous phase, while the semivolatile priority pollutants are removed into the organic phase. Studies were conducted to determine the cleanup efficiencies that could be expected using various solvent systems. A fish tissue mixture was prepared by combining approximately equal weights of scallop, salmon fillets and catfish fillets obtained from a local fish market. The combined fish tissues were ground without addition of water using a Brinkmann Polytron. The ground fish tissue was divided into 25-gram aliquots, placed in screw-capped glass jars and frozen. This ground fish tissue mixture was homogenized in acetonitrile and the acetonitrile was isolated after centrifuging. Residue weight measurements were taken by placing 100-µL aliquots of the acetonitrile extract onto tared foil pans and placing the pans under a heat lamp. The distance of the pans from the heat lamp, heating time, and heating intensity were carefully controlled and reproducible. The material remaining in

the pans after evaporation of the solvent, defined as the fish lipid material, was weighed. Triplicate residue weight measurements demonstrated that the acetonitrile extract contained 6.7 mg of fish lipid per milliliter of acetonitrile. A 0.75-mL aliquot of the acetonitrile extract containing 5 mg of fish lipid material was placed in a 15-mL conical centrifuge tube containing 5 mL of 2 percent sodium sulfate adjusted to pH 4 and 1.5 mL of one of the organic solvent systems. The tube was capped and tumbled end-over-end for one hour. Triplicate residue weight measurements were taken on 100-µL aliquots of the organic phase using the procedure described earlier; these residue weight data were used to determine the amount of lipid material removed into the organic phase. All partitioning experiments were conducted in triplicate.

Comparison of Tumbling and Separatory Funnel Partitioning Procedures

Two acetonitrile partitioning procedures were examined. The first procedure used separatory funnels for the partitioning step. The second procedure used tumbling to perform the same function. The previously described ground fish tissue mixture was extracted by homogenizing the tissue in 3 mL of acetonitrile per gram of fish tissue extracted. Homogenization was done using a Brinkmann Polytron homogenizer. The homogenate was centrifuged and the supernatant removed to a graduated cylinder. The homogenization procedure was repeated twice, and the supernatants were combined in the graduated cylinder. The combined acetonitrile extract was spiked with selected priority pollutant compounds at a level representing 5 µg of each compound per gram of fish tissue. This stock spiked fish extract was used to perform all fish analysis procedure evaluation experiments described below.

The fish extraction procedure using acetonitrile partitioning in separatory funnels was examined by adding 180 mL of the spiked acetonitrile fish extract, representing 20 grams of fish sample spiked with selected priority pollutant compounds at the 5 µg/g level, to a separatory funnel containing 1300 mL of 2 percent sodium sulfate that had been adjusted to pH<2. The aqueous mixture was partitioned three times with 60 mL each of 50 percent MTBE in petroleum ether. The contents of the separatory funnel were vigorously shaken for 2 minutes during each partitioning step. The

organic extracts were combined, dried over sodium sulfate, and concentrated to 2 mL using Kuderna-Danish (K-D) techniques. The volume of the extract was adjusted to 10 mL by the addition of 8 mL of 50 percent methylene chloride in hexane. Half of the extract, representing 10 g of fish sample spiked with selected priority pollutant compounds at the 5 µg/g level, was cleaned up by GPC using conditions reported later in this report. The GPC fraction containing the priority pollutant compounds was concentrated to 5 mL using K-D equipment and techniques and analyzed by capillary column GC-FID to determine recoveries of the spiked priority pollutant compounds. each sample was prepared in triplicate.

The fish extraction procedure using acetonitrile partitioning by tumbling was examined by adding 225 mL of the spiked acetonitrile fish extract, representing 25 grams of fish sample spiked with selected priority pollutant compounds at the 5 µg/g level, to a 2.5-liter tumbling jar containing 1500 mL of 2 percent sodium sulfate and 1.5 percent monobasic potassium phosphate (pH 4) and 450 mL of 50 percent MTBE in petroleum ether. The contents of the bottle were tumbled end-over-end for 1 hour. The organic extract was measured to have a volume of 420 mL; as much of the organic extract as could be isolated was removed from the bottle and placed in an Erlenmeyer flask containing sodium sulfate. A 360-mL aliquot of the dried organic extract, representing 21.4 grams of fish sample spiked with the selected priority pollutant compounds at the 5 µg/g level, was removed and concentrated to 2 mL using K-D techniques. The volume of the extract was adjusted to 10 mL by the addition of 8 mL of 50 percent methylene chloride in hexane. The fish extract was then processed by GPC, reconcentrated, and analyzed by GC-FID as described in the above paragraph. Each sample was prepared in triplicate.

At the request of the EPA Project Officer, 50 percent MTBE in hexane was also investigated as a possible substitute for the 50 percent MTBE in petroleum ether used in the above studies. Hexane is widely used for pesticide analyses and is specified in the EPA Interim Method. The tumbling acetonitrile partitioning procedure was used as described earlier, except hexane was substituted for the petroleum ether used in those studies.

It was necessary to determine if any observed recovery losses occurred during the acetonitrile partitioning step, or if they occurred during the GPC cleanup. Triplicate GPC spike blank samples were prepared containing 100 µg of each of the selected priority pollutant compounds in 10 mL of 50 percent methylene chloride in hexane. Half of each GPC spike blank sample, 5 mL, was injected onto the GPC system and the appropriate fraction was analyzed by GC-FID.

Gel Permeation Chromatography

GPC studies were conducted to evaluate the efficiency of the method for removing fish lipid material from the various semivolatile organic priority pollutant compounds. In order to conduct the studies, fish lipid material was isolated from the previously described ground fish tissue mixture. The ground fish tissue was divided into 25-gram aliquots, placed in screw-capped glass jars and frozen. Fish lipid material was extracted from the fish by homogenizing the fish in the presence of methylene chloride and magnesium sulfate. Methylene chloride, 200 mL, and 25 grams of magnesium sulfate were placed in a 600-mL beaker. The mixture was homogenized using a Polytron and 10 grams of fish tissue were slowly added. Additional methylene chloride was added to the beaker to compensate for that lost during the homogenization procedure. The mixture was centrifuged, 168 mL of the methylene chloride layer was removed, and concentrated to 10 mL using the standard K-D equipment and techniques. The resultant extract contained extractable material representing 8.4 grams of fish tissue. Triplicate residue weights were taken on the extracts using the procedure described earlier. The extracts contained 25 mg of fish lipid material per milliliter of extract. The extraction procedure was performed five times to assure sufficient lipid material to conduct the GPC studies.

GPC conditions used were as follows:

Column:	600 mm x 25 mm I.D. packed with 70 grams of BioBeads S-X3 swelled in 50 percent methylene chloride in hexane overnight
Solvent:	50 percent methylene chloride in hexane
Flow rate:	5 mL/min upward flow
Sample volume:	5 mL

The 50 percent methylene chloride in hexane solvent was chosen because past experience and literature references indicated that nonpolar solvents such as hexane or cyclohexane yield superior resolution of priority pollutant compounds from fish lipid material.[1] Use of 100 percent hexane or cyclohexane leads to precipitation problems during sample extract concentration steps before and after the GPC cleanup procedure. Use of the nonpolar solvent in combination with methylene chloride allows increased resolution capabilities while avoiding precipitation problems.

Two 5-mL aliquots of the fish lipid extract containing 125 mg each of fish lipid material were injected into the GPC system and fractions were collected each minute. Residue weight data were tabulated for each fraction to be used as an indication of the elution characteristics of the lipid material. The elution characteristics of the semivolatile organic priority pollutant compounds were also studied by injecting solutions prepared from the priority pollutant mixtures available from Supelco (base/neutral 1, base/neutral 2, base/neutral 3, base/neutral 4, and phenols). These solutions contained the priority pollutant compounds at the 20 g/mL concentration level; a 5-mL aliquot of each solution, containing 100 g of each of the priority pollutant compounds, was injected onto the GPC system. Fractions were collected every minute from the time of sample injection until 45 minutes after sample injection. Each fraction was concentrated to 1 mL using K-D techniques and analyzed by capillary column GC-FID. Compound identifications were made on the basis of retention time.

A standard solution containing corn oil, dioctylphenylphosphonate, 2,6-dibromophenol, and sulfur in 50 percent methylene chloride in hexane was designed for use in GPC calibration. The peaks that are detected when this solution is analyzed on the GPC system are used as markers to determine where GPC fraction collection should be initiated and terminated.

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[1] J. Assoc. Off. Anal. Chem., 55(6), 1360-2 [1972]

The draft fish analysis method was submitted to the EPA Project Officer after completion of preliminary laboratory experimentation and is included as Appendix A of this report.

INSTRUMENT RANGE STUDIES AND PRELIMINARY METHOD EVALUATION

Instrument Range Studies

Instrument detection limits (IDLs) and instrument range were determined. Solutions of the priority pollutant compounds were analyzed by GC-MS using the following conditions:

Instrument:	Finnigan Series 5100 GC/MS system
Column:	30 m x 0.25 mm I.D. SDB-5 fused silica capillary column
Injection Port Temp:	300°C
Transfer Line Temp:	300°C
Oven Conditions:	60°C to 300°C at 4°C/min; hold at 300°C for 10 min
Sample:	1 µL splitless; turn on split 45 sec after injection
Carrier Gas:	Helium; 40 cm/min linear velocity

Extracted ion current profiles (EICPs) were generated for each compound using the primary electron impact ion specified in EPA Method 625. Signal-to-noise ratios (S/N) were determined using the peak heights of the priority pollutant compounds and recovery compounds in each EICP; solutions containing the priority pollutant compounds and recovery standards at concentrations of 0.1, 0.5, 1.0, 5.0, 10 and 20 µg/mL were analyzed. If the S/N was between 2 and 10, the IDL for the compound, defined as the concentration of the compound in µg/mL in solution that yielded a S/N of 5, was calculated. All measurements were taken in triplicate and the average used to calculate the IDLs.

Solutions of the priority pollutant compounds and recovery standards at the 0.1, 0.3, 0.5, 1.0, 3.0, 5.0, 10, 20, 50, and 100 µg/mL concentration levels were then analyzed in triplicate and the ratios of the resultant EICP peak areas to the EICP peak area of the d_{10}-phenanthrene internal standard (present at the 20 µg/mL level in all solutions) were calculated.

Preliminary Method Evaluation

The draft method was performed as written as a preliminary evaluation of the method. The draft method is included in this report as Appendix A. Preliminary method evaluation studies were conducted using catfish fillets obtained from a local fish market; the tissue was ground without addition of water using a Polytron. Aliquots of the ground catfish, 25 grams each, were placed in 200-mL centrifuge tubes and spiked with 50 to 1000 µg of each of the priority pollutant compounds. Additions of these levels of priority pollutant compounds correspond to priority pollutant compound concentrations in fish tissue ranging from 2 to 40 µg/g. Compound spiking levels were approximately 20 times the calculated IDLs. Eight spiked and one unspiked fish tissue samples were processed immediately after spiking. One of the spiked fish tissue samples was lost during sample processing. The resultant fish extracts were analyzed by GC-MS and recoveries were determined using response factors generated from a standard prepared using the original priority pollutant spiking solution.

At the request of the EPA Project Officer, attempts were made to establish the effectiveness of the method for determination of priority pollutant compounds that have been incorporated into the fish tissue. Obtaining a fish matrix with established priority pollutant compound content was not possible. In an attempt to simulate this situation, the fish was spiked with known levels of priority pollutant compounds and then stored for a 24-hour period to allow incorporation of the compounds into the tissue. Fish samples, specifically eight 25-gram aliquots of ground catfish fillets as used in the preliminary method evaluation, were spiked with the semivolatile priority pollutant compounds; the spiking levels were identical to those used in the preliminary method evaluation. The compounds were added directly to the ground fish tissue in approximately 200 µL of MTBE. The samples were agitated, covered, and stored refrigerated for 24 hours prior to extraction with acetonitrile and subsequent processing. One spiked fish sample was lost during sample processing. Recoveries were determined by GC-MS using the historical response factor

data given in Table 17 for compound quantification. These analyses were performed approximately three weeks after the historical response factor data were generated.

METHOD RANGE AND METHOD DETECTION LIMIT STUDIES

Method range and method detection limits studies were conducted to determine how well the analysis procedure performed in fish over the GC-MS instrument range as well as to determine the compound detection limits in a fish matrix. The fish analysis method was performed as written. Method range and method detection limit studies were conducted using a fish matrix consisting of equal parts of ground catfish, scallops, and salmon obtained from a local fish market. The tissue was ground without addition of water using a Polytron. Fish tissue aliquots, 25 grams each, were spiked at one of five concentrations levels with the priority pollutant compounds. The spiking levels were chosen such that they spanned the calculated GC-MS instrument range. Eight replicate fish tissue samples were prepared at each concentration level; two unspiked tissue samples were analyzed as method blanks.

MATRIX VALIDATION STUDIES

Matrix validation studies were conducted as a comparison to the method range studies in order to determine if varying fish matrices might effect the analytical results obtained. The fish analysis method was performed as written. The matrix validation studies were conducted using minnows obtained from a local bait store. The whole minnows were ground without addition of water using a Polytron. Fish tissue aliquots, 25 grams each, were spiked at one of two concentrations levels with the priority pollutant compounds. The spiking levels were chosen at the high and low ends of the calculated GC-MS instrument range. Eight replicate fish tissue samples were prepared at each concentration level; two unspiked tissue samples were analyzed as method blanks.

SECTION 5

RESULTS AND DISCUSSION

PRELIMINARY LABORATORY EXPERIMENTATION

Acetonitrile Extraction and Partitioning

Phase volumes observed after acetonitrile partitioning are given in Table 6. Percent recoveries (R) of selected priority pollutant compounds after acetonitrile partitioning conducted without the presence of fish lipid material were calculated using the following equation:

$$R = \frac{Cs \times Vs \times 100}{Ao}$$

where Cs is the concentration of the compound in the organic phase as determined by GC-FID, Vs is the volume of the organic phase given in Table 6, and Ao is the amount of the compound spiked into the partitioning mixture. Results of acetonitrile partitioning studies conducted without the presence of fish lipid material are given in Table 7. As expected, recoveries of relatively nonpolar compounds such as 1,4-dichlorobenzene, and di-n-butyl phthalate were generally greater than 90 percent when petroleum ether or hexane were used as the organic phase. However, recoveries of polar compounds, especially phenol and 4-nitrophenol were low. Use of the more polar extraction solvents, specifically MTBE and methylene chloride, significantly increased the recoveries of the more polar priority pollutant compounds from the aqueous phase. The extremely polar 4-nitrophenol was not recovered from water using petroleum ether or hexane as the extraction solvent; recoveries of 4-nitrophenol increased to 71 percent when MTBE was substituted for the petroleum ether.

Standard EPA extraction procedures use three 60-mL separatory funnel extractions of a one liter aqueous sample. This standard extraction procedure is also used in the EPA Interim Method. Various calculations were performed in order to compare the expected extraction efficiencies using the separatory funnel extraction technique to the single tumbling

TABLE 6. VOLUMES OF ORGANIC AND AQUEOUS PHASES AFTER ACETONITRILE PARTITIONING

Solvent System (a)	Phase Volumes, mL (b) Organic (Vs)	Aqueous (Vw)	VCF (c)
A	139	573	0.93
B	141	576	0.94
C	185	540	1.23
D	165	553	1.10
E	153	565	1.02
F	140	580	0.93
G	140	572	0.93
H	150	570	1.00

(a) A = 100% petroleum ether
B = 100% MTBE
C = 100% methylene chloride
D = 50% methylene chloride in petroleum ether
E = 25% methylene chloride in petroleum ether
F = 50% MTBE in petroleum ether
G = 25% MTBE in petroleum ether
H = 100% hexane

(b) Average of duplicate measurements.

(c) VCF = Volume change factor; change in volume of organic phase after partitioning; VCF = Vs/150

TABLE 7. RESULTS FROM ACETONITRILE PARTITIONING STUDIES USING TUMBLING

Compound	Percent Recovery using Given Solvent System (a)(b)							
	A	B	C	D	E	F	G	H
Phenol	3	101	88	68	47	75	49	4
2-Chlorophenol	36	90	83	78	74	81	71	40
1,4-Dichlorobenzene	87	96	89	90	93	93	94	91
Nitrobenzene	74	96	92	92	94	91	86	76
2-Nitrophenol	67	87	86	84	86	84	79	73
2,4-Dimethylphenol	41	99	93	91	89	94	88	41
Bis(2-chloroethoxy)methane	50	74	71	71	72	68	62	66
2,4-Dichlorophenol	57	88	81	80	81	86	82	65
Naphthalene	97	106	100	102	105	104	103	91
3-Methyl-4-chlorophenol	34	96	90	87	84	92	86	35
2,4,6-Trichlorophenol	71	81	78	78	79	81	80	84
2,6-Dinitrotoluene	69	92	90	89	90	89	84	75
4-Nitrophenol	0	75	52	42	22	55	25	0
2,4-Dinitrotoluene	60	90	85	85	84	86	77	66
2-Methyl-4,6-dinitrophenol	33(c)	11	53	57	54	59	54	43
Pentachlorophenol	58	62	56	59	57	63	61	82
Di-n-butyl phthalate	88	97	87	92	88	91	90	88
Chrysene	85	95	80	87	40	72	86	77

(a) A = 100% petroleum ether
B = 100% MTBE
C = 100% methylene chloride
D = 50% methylene chloride in petroleum ether
E = 25% methylene chloride in petroleum ether
F = 50% MTBE in petroleum ether
G = 25% MTBE ether in petroleum ether
H = 100% hexane

(b) Data are the average of three replicate analyses; percent relative standard deviations were generally less than 10 percent.

(c) High percent relative standard deviation indicates data point not reliable.

extraction technique involving 300 mL of solvent per liter of aqueous phase used for these studies. Using the recovery data presented in Table 7 obtained from the acetonitrile partitioning experiments, distribution coefficients were calculated for each of the analytes.

The partition coefficient (Kd) for a compound is defined as the ratio of the concentration of the compound in the organic phase (Cs) to the concentration of the compound in the aqueous phase (Cw). Kd can be described in terms of the amount of the compound in the organic phase (As), the amount of the compound originally spiked into the partitioning mixture (Ao) and the volumes of the organic (Vs) and aqueous (Vw) phases:

$$Kd = \frac{Cs}{Cw} = \frac{As \times Vw}{Aw \times Vs} = \frac{As \times Vw}{(Ao - As) \times Vs}$$

If the relationship between compound concentrations in organic phase and percent recovery is considered, R = (100 x As)/Ao, the following equation can be used to calculate Kd:

$$Kd = \frac{Vw}{Vs \times [(100/R)-1)]}$$

Distribution coefficients calculated as described using the percent recovery data given in Table 7 are presented in Table 8. Kd could not be calculated if the recovery listed in Table 7 was greater than or equal to 100 percent. In these cases, a recovery of 99 percent was used for calculations of the compound Kd.

The Kd values given in Table 8 were used to calculate recoveries expected to be obtained from extracting a one-liter sample with three 60-mL aliquots of organic solvent. The percent recovery (R) of the compound into the combined organic phases was be calculated using the following equation:

$$R = 100 \times \left[1 - \left[\frac{1}{(Kd \times Vr) + 1} \right]^3 \right]$$

TABLE 8. PARTITION COEFFICIENTS CALCULATED FROM ACETONITRILE TUMBLING DATA

Compound	Partition Coefficient in Given Solvent System (a)(b)							
	A	B	C	D	E	F	G	H
Phenol	0.15	404.43	21.04	7.22	3.28	12.23	4.00	0.14
2-Chlorophenol	2.30	36.47	14.04	11.91	10.34	17.70	10.06	2.52
1,4-Dichlorobenzene	27.65	88.00	22.57	30.21	48.80	56.60	64.19	37.67
Nitrobenzene	11.77	104.19	33.38	41.12	60.17	41.62	25.64	11.92
2-Nitrophenol	8.34	27.91	17.81	18.19	22.25	21.53	15.18	10.13
2,4-Dimethylphenol	2.83	747.44	37.24	32.19	30.67	66.79	29.29	2.69
Bis(2-chloroethoxy)methane	4.10	11.51	7.16	8.35	9.38	8.79	6.77	7.49
2,4-Dichlorophenol	5.45	31.33	12.86	13.46	15.31	25.78	18.54	7.04
Naphthalene	139.83	404.43	288.97	331.80	365.59	410.14	404.49	38.38
3-Methyl-4-chlorophenol	2.16	95.93	25.60	23.01	19.51	44.89	24.61	2.07
2,4,6-Trichlorophenol	10.09	16.97	10.36	11.57	14.20	18.18	15.85	19.87
2,6-Dinitrotoluene	9.12	44.72	26.36	27.92	34.07	32.47	21.55	11.11
4-Nitrophenol	(c)	12.12	3.21	2.45	1.03	5.16	1.39	(c)
2,4-Dinitrotoluene	6.29	35.59	17.09	18.77	19.00	24.95	13.46	7.54
2-Methyl-4,6-dinitrophenol	1.99	0.53	3.34	4.53	4.34	6.02	4.75	2.88
Pentachlorophenol	5.74	6.54	3.65	4.84	4.91	7.02	6.51	17.54
Di-n-butyl phthalate	29.89	119.41	19.21	38.15	28.15	40.28	38.86	26.93
Chrysene	22.66	79.06	12.05	23.14	2.48	10.51	24.25	12.55

(a) A = 100% petroleum ether
B = 100% MTBE
C = 100% methylene chloride
D = 50% methylene chloride in petroleum ether
E = 25% methylene chloride in petroleum ether
F = 50% MTBE in petroleum ether
G = 25% MTBE ether in petroleum ether
H = 100% hexane

(b) Partition coefficient (Kd) calculated using recovery data presented in Table 7 and phase volume data presented in Table 6. In cases where recoveries were greater than 100 percent, a value of 99 percent recovery was used to calculated the Kd.

(c) Recovery of 0 percent did no allow calculation of Kd.

where Vr is the ratio of the volume of the organic phase (Vs) to the volume of the aqueous phase (Vw). The derivation of the above equation and a thorough discussion of phase equilibria theory are presented in Chapter 15 of the book entitled "Chemical Separations and Measurements."[2]

Expected recovery data using the standard separatory funnel extraction procedure are presented in Table 9. Several assumptions were made while making these calculations:

(1) It is assumed that equilibration is as complete after shaking for two minutes in the separatory funnel as it is in one hour using the tumbling procedure.

(2) The volume of the organic phase (Vs) is increased or decreased after each contact with the aqueous phase, and the change in Vs is proportionally the same as the change observed during the acetonitrile partitioning studies as presented in Table 6. The volume change factor (VCF) was used to calculate the volume of the organic phase (Vs), using the equation: Vs = 60 x VCF.

(3) The volume of the aqueous phase (Vw) remains essentially constant at one liter.

The calculated differences in recoveries obtained using the two extraction methods are presented in Table 10. The single extraction technique is expected to result in comparable extraction efficiency for most of the priority pollutant compounds as evidenced by calculated recovery differences of less than seven percent. Additional advantages of the single extraction method are the comparative ease of sample handling and the lessened chance of variable results due to differences in the effectiveness of separatory funnel shaking by different workers.

Since the EPA Interim Method specifies that the aqueous phase be adjusted to pH<2 prior to extraction, recoveries of priority pollutant compounds into hexane, specifically the acidic priority pollutant compounds, were also studied at pH 1.5 for comparison to the abovementioned data obtained when the aqueous layer was adjusted to pH 4.0. Recovery data obtained using hexane as the organic phase and using water adjusted to

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[2] Peters, D. G., J. M. Hayes, and G. M. Hieftje. Chemical Separations and Measurements. W. B. Saunders Company, Philadelphia, Pennsylvania, 1974. Chapter 15.

TABLE 9. CALCULATED RESULTS FROM ACETONITRILE PARTITIONING STUDIES USING SEPARATORY FUNNELS

Compound	Percent Recovery using Given Solvent System (a)(b)							
	A	B	C	D	E	F	G	H
Phenol	2	100	94	69	42	79	45	2
2-Chlorophenol	30	96	88	82	77	87	74	34
1,4-Dichlorobenzene	94	100	95	96	98	99	99	97
Nitrobenzene	78	100	98	98	99	97	93	80
2-Nitrophenol	68	94	92	91	92	91	84	76
2,4-Dimethylphenol	35	100	98	97	96	99	95	36
Bis(2-chloroethoxy)methane	46	78	72	73	74	70	62	67
2,4-Dichlorophenol	55	95	87	85	86	93	88	65
Naphthalene	100	100	100	100	100	100	100	97
3-Methyl-4-chlorophenol	29	100	96	94	91	98	93	30
2,4,6-Trichlorophenol	74	87	82	82	85	88	85	91
2,6-Dinitrotoluene	71	98	96	96	97	96	91	78
4-Nitrophenol	0	79	47	36	17	53	20	0
2,4-Dinitrotoluene	59	96	91	91	90	93	81	67
2-Methyl-4,6-dinitrophenol	27 (c)	8	48	54	51	58	51	38
Pentachlorophenol	56	61	51	56	55	63	61	88
Di-n-butyl phthalate	95	100	93	98	95	97	97	94
Chrysene	91	99	85	94	35	75	92	81

(a) A = 100% petroleum ether
B = 100% MTBE
C = 100% methylene chloride
D = 50% methylene chloride in petroleum ether
E = 25% methylene chloride in petroleum ether
F = 50% MTBE in petroleum ether
G = 25% MTBE ether in petroleum ether
H = 100% hexane

(b) Calculated recoveries using Kd values shown in Table 8.

(c) High percent relative standard deviation indicates data point not reliable.

TABLE 10. CALCULATED RECOVERY DIFFERENCES BETWEEN TUMBLING AND SEPARATORY FUNNEL ACETONITRILE PARTITIONING TECHNIQUES

Compound	Percent Recovery using Given Solvent System (a)(b)							
	A	B	C	D	E	F	G	H
Phenol	1	1	-6	-1	5	-4	4	1
2-Chlorophenol	6	-7	-5	-4	-3	-6	-3	5
1,4-Dichlorobenzene	-7	-4	-6	-6	-5	-5	-5	-6
Nitrobenzene	-4	-3	-6	-6	-5	-6	-7	-4
2-Nitrophenol	-1	-7	-6	-6	-7	-7	-5	-3
2,4-Dimethylphenol	5	-1	-5	-6	-7	-5	-7	5
Bis(2-chloroethoxy)methane	4	-4	-1	-2	-3	-2	0	-1
2,4-Dichlorophenol	2	-7	-5	-5	-6	-7	-6	0
Naphthalene	-3	6	0	2	5	4	3	-6
3-Methyl-4-chlorophenol	6	-4	-6	-6	-6	-6	-7	6
2,4,6-Trichlorophenol	-3	-6	-4	-4	-5	-6	-6	-7
2,6-Dinitrotoluene	-2	-6	-6	-6	-6	-7	-7	-4
4-Nitrophenol	0	-4	5	6	5	2	5	0
2,4-Dinitrotoluene	1	-7	-6	-6	-6	-7	-5	-1
2-Methyl-4,6-dinitrophenol	6	3	5	3	3	1	3	5
Pentachlorophenol	2	1	4	3	3	0	1	-6
Di-n-butyl phthalate	-7	-3	-6	-6	-7	-6	-6	-7
Chrysene	-7	-4	-5	-6	6	-3	-7	-5

(a) A = 100% petroleum ether
B = 100% MTBE
C = 100% methylene chloride
D = 50% methylene chloride in petroleum ether
E = 25% methylene chloride in petroleum ether
F = 50% MTBE in petroleum ether
G = 25% MTBE ether in petroleum ether
H = 100% hexane

(b) The value given S-T, where S is the percent recovery using a single extraction with 300 mL of solvent per 100 mL of water, and T is the percent recovery using triplicate extractions with 60 mL of solvent for each extraction per 1000 mL of water. Thus, a positive value indicates that the single extraction approach gives a higher percent recovery.

pH 1.5 and 4.0 are presented in Table 11. Recoveries of acidic priority pollutant compounds did not improve significantly when the pH of the aqueous phase was reduced from 4.0 to 1.5.

The priority pollutant recovery data presented in Table 7 indicate that MTBE should be substituted for petroleum ether in the acetonitrile partitioning procedure. However, priority pollutant recoveries are not the only factor that must be considered. The acetonitrile partitioning procedure is designed to remove as much lipid material as possible from the priority pollutants extracted from the fish. Presumably the acetonitrile and the majority of the fish lipid material remain in the aqueous layer, as is the case when petroleum ether or hexane are used as the extraction solvent. However, substitution of more polar extraction solvents for the petroleum ether can lead to extraction of larger amounts of the acetonitrile into the organic phase. Presumably, the effectiveness of the acetonitrile partitioning procedure for the removal of lipid material from the organic phase would be decreased if the acetonitrile went into the organic phase. Acetonitrile levels were determined in the organic extracts obtained from the acetonitrile partitioning procedure studies by analyzing those extracts by packed column GC-FID. Percentages of acetonitrile found in those extracts are presented in Table 12. As expected, acetonitrile content of the organic phase increased as the polarity of the organic phase was increased. However, although use of 50 percent MTBE in petroleum ether instead of 100 percent petroleum ether as the extraction solvent dramatically increased recoveries of polar priority pollutant compounds, acetonitrile content of the organic phase was only increased by one to five percent. Presumably, 50 percent MTBE in petroleum ether extraction solvent could be used to increase recoveries of polar compounds without significantly decreasing the efficiency of lipid removal.

Acetonitrile Partitioning Cleanup Efficiency Studies

Calculated cleanup efficiencies, reported as the percent of lipid material remaining in the aqueous phase are reported in Table 13. In general, 66 to 77 percent of the lipid material was not extracted into the organic phase during the partitioning procedure, regardless of the extraction solvent used.

TABLE 11. RECOVERIES OF SELECTED PRIORITY POLLUTANTS AFTER ACETONITRILE PARTITIONING AT pH 1.5 AND pH 4.0 USING HEXANE AS THE EXTRACTION SOLVENT

Compound	Percent Recovery at Given pH (a)	
	pH 4.0	pH 1.5
Phenol	4	4
2-Chlorophenol	40	38
1,4-Dichlorobenzene	91	91
Nitrobenzene	76	76
2-Nitrophenol	73	73
2,4-Dimethylphenol	41	38
Bis(2-chloroethoxy)methane	66	63
2,4-Dichlorophenol	65	63
Naphthalene	91	91
3-Methyl-4-chlorophenol	35	45
2,4,6-Trichlorophenol	84	83
2,6-Dinitrotoluene	75	73
4-Nitrophenol	0	0
2,4-Dinitrotoluene	66	66
2-Methyl-4,6-dinitrophenol	43	63
Pentachlorophenol	82	82
Di-n-butyl pthalate	88	88
Chrysene	77	85

(a) Data are the average of three replicate analyses; percent standard deviations were generally less than 10 percent.

TABLE 12. ACETONITRILE CONTENT OF ORGANIC PHASES AFTER PARTITIONING

Solvent System	Percent Acetonitrile in Organic Phase
100% Petroleum ether	1
100% Methyl t-butyl ether	17
100% Methylene chloride	33
50% Methylene chloride in petroleum ether	26
25% Methylene chloride in petroleum ether	12
50% Methyl t-butyl ether in petroleum ether	5
25% Methyl t-butyl ether in petroleum ether	3

TABLE 13. LIPID REMOVAL EFFICIENCY OF ACETONITRILE PARTITIONING PROCEDURE

Extraction Solvent	Cleanup Efficiency (a)
100% petroleum ether	70
100% methyl t-butyl ether	70
100% methylene chloride	72
50% methylene chloride in petroleum ether	74
25% methylene chloride in petroleum ether	69
50% methyl t-butyl ether in petroleum ether	68
25% methyl t-butyl ether in petroleum ether	70

(a) Data are the average of three replicate analyses; cleanup efficiency is defined as the percent of lipid material not carried over into the organic extract containing the priority pollutant compounds.

Summary of Extraction and Partitioning Studies Results

There are several factors that must be considered when choosing the best solvent for use in the acetonitrile partitioning procedure. First, the solvent must yield acceptable recoveries of even the more polar priority pollutant compounds such as the phenols. From that standpoint, nonpolar solvents such as hexane or petroleum ether are clearly unacceptable. However, if more polar extraction solvents such as methylene chloride and MTBE are introduced, recoveries of polar priority pollutants are markedly improved. The effect of the extraction solvent on the cleanup efficiency achieved during the acetonitrile partitioning procedure must also be considered. Lipid removal studies indicate that cleanup efficiencies did not vary significantly regardless of the polarity of the extraction solvent. A third consideration is the amount of acetonitrile that will remain in the organic phase. Most of the acetonitrile must be removed from the extract prior to introduction onto the GPC, and acetonitrile is difficult to remove using conventional concentration techniques. Nonpolar extraction solvents such as petroleum ether contain only a small percentage of acetonitrile after the partitioning step; however, nonpolar extraction solvents do not yield acceptable recoveries of polar priority pollutant compounds. Polar extraction solvents such as methylene chloride or MTBE yield acceptable recoveries of priority pollutant compounds, but contain 20 to 30 percent acetonitrile after the partitioning step. The optimum situation is obtained when 50 percent MTBE in petroleum ether is used as the extraction solvent. Use of 50 percent MTBE in petroleum ether yields acceptable recoveries of polar priority pollutant compounds and contains only five percent acetonitrile after the partitioning step.

Comparison of Tumbling and Separatory Partitioning Procedures

Recoveries of priority pollutants in the presence of fish lipid using the separatory funnel and tumbling acetonitrile partitioning techniques are given in Table 14. In general, percent recoveries achieved using the tumbling partitioning technique with 50 percent MTBE in petroleum ether organic solvent were within 10 percent of recoveries observed from the spiked blank sample which was only processed by GPC. However, recover-

TABLE 14. RESULTS FROM COMPARISON OF TUMBLING AND SEPARATORY FUNNEL ACETONITRILE PARTITIONING STUDIES

No.	Compound	Recovery From Given Sample, %(a)								
		Spiked Blank(b)		Sep Funnel(d)		Tumbling(d)		Tumbling(e)		Sep Funnel Theor. (g)
		Avg.	%RSD	Avg.	%RSD	Avg.	%RSD	Avg.	%RSD	
1.	Phenol	76	9	52	8	77	10	54	4	79
2.	2-Chlorophenol	83	14	87	5	100	9	66	2	87
3.	1,4-Dichlorobenzene	82	16	95	5	106	9	48	3	99
4.	Nitrobenzene	86	13	92	6	99	8	72	2	97
5.	2-Nitrophenol	88	8	83	5	91	9	79	1	91
6.	2,4-Dimethylphenol	91	2	81	5	86	7	93	3	99
7.	Bis-(2-Chloroethoxy)methane	90	8	78	5	88	8	80	1	70
8.	2,4-Dichlorophenol	89	6	85	6	94	8	89	3	93
9.	Naphthalene	88	7	87	6	98	8	80	3	100
10.	4-Chloro-3-methylphenol	98	4	75	4	81	7	99	1	98
11.	2,4,6-Trichlorophenol	94	2	79	4	89	6	94	2	88
12.	2,6-Dinitrotoluene	102	6	79	3	88	7	102	1	96
13.	4-Nitrophenol	33(f)	81	69	5	104	6	(c)	—	53
14.	2,4-Dinitrotoluene	103	4	79	4	86	6	104	7	93
15.	2-Methyl-4,6-dinitrophenol	106	6	54	7	55	10	64	6	58
16.	Pentachlorophenol	103	13	94	4	105	8	(c)	—	63
17.	Dibutylphthalate	89	2	(c)	—	(c)	—	(c)	—	97
18.	Chrysene	91	8	103	8	107	11	122	7	75

(a) Data based on triplicate analyses.
(b) Data represent recoveries obtained from standards run through the GPC cleanup only.
(c) Presence of interferences precluded quantification of compound.
(d) Partitioning solvent used was 50 percent MTBE in petroleum ether.
(e) Partitioning solvent used was 50 percent MTBE in hexane.
(f) Data point not reliable.
(g) Calculated recovery presented in Table 9 for separatory funnel method using solvent system F, 50 percent MTBE in petroleum ether.

ies of the extremely polar 2-methyl-2,6-dinitrophenol were lowered by approximately 50 percent when the tumbling partitioning procedure was used. Lowered recoveries of polar compounds was not unexpected. Recoveries of neutral compounds after separatory funnel partitioning were approximately equivalent to those observed after tumbling partitioning. However, recoveries of polar compounds such as the phenols were generally decreased by 10 to 20 percent when the separatory funnel procedure was substituted for the tumbling procedure. On the average, recoveries of compounds using the separatory funnel partitioning method were four percent lower than theoretical recoveries calculated on the basis of partition coefficients as presented in Table 9. The close correlation between theoretical and actual recoveries indicates that the phase equilibria model used for the calculations presented in Table 9 was suitable. Lower actual recoveries as compared to the theoretical estimates may indicate that a true equilibrium is not obtained when the separatory funnel is shaken for two minutes. Recoveries achieved using the tumbling technique with 50 percent MTBE in hexane organic solvent were lower than those observed using any other partitioning conditions presumably because of the more rigorous conditions required for concentration of large volumes of hexane.

These recovery data indicate that the tumbling procedure can be substituted for the more commonly used separatory funnel partitioning techniques; use of the tumbling procedure greatly reduces the amount of time and glassware required for the extraction and provides comparable or superior recovery results. Use of hexane for petroleum ether as part of the organic solvent used in the partitioning procedure is not advised because of potential recovery losses during solvent concentration procedures.

Residue weight measurements taken on the extracts obtained after separatory funnel and tumbling acetonitrile partitioning showed that approximately three-fourths of the lipid material was removed from the fish extract when the tumbling acetonitrile partitioning procedure was used, but only half of the the lipid material was removed from the fish extract using the separatory funnel acetonitrile partitioning procedure. These data may indicate that lipid removal is more efficient with the aqueous phase at pH 4 than with the aqueous phase at pH 1.5. This conclusion was not confirmed by further studies.

Gel Permeation Chromatography

The elution characteristics of the semivolatile organic priority pollutants, specifically the phthalates, polynuclear aromatic hydrocarbons (PAHs), substituted benzenes, phenols, and other miscellaneous compounds, are graphically demonstrated in Figures 1-5. Approximately 99.3 percent of this lipid material is removed prior to the elution of any of the priority pollutant compounds. These studies indicated that greater than 99 percent of the lipid material could be removed from the fish extract without loss of even the long-chain phthalates. Recoveries of the priority pollutants from the GPC were not determined, however, past experience with the system indicates that quantitative recoveries of all of the analytes can be expected.

The chromatogram resulting from the GPC analysis of the GPC calibration solution is shown in Figure 6. Each of the four components of the calibration mixture is labeled in the chromatogram. The absolute retention time of each of the components depends upon the individual GPC column being used and depends upon such factors as column size and packing density. The retention time of each component, however, does give an indication of general retention time trends of various compounds groups. For instance, the elution of corn oil gives a rough idea of where the bulk of the fish lipid material will elute. The elution time of dioctylphenyl-phosphonate indicates where long-chain phthalates will elute, although the dioctylphenyl phosphonate elutes slightly earlier than the priority pollutant octyl phthalates. Sulfur elutes later than any organic compound yet identified. After consideration of the fish lipid and priority pollutant elution data, guidelines were set up to define when the GPC fraction should be collected. Initially, fraction collection was initiated at the time as indicated by the valley between the corn oil and dioctyl-phenyl phosphonate peaks and was terminated after elution of the sulfur peak as indicated in Figure 6. This sample collection procedure was suitable during the preliminary method evaluation studies which used catfish fillets as the fish matrix. During method validation and matrix validation studies discussed later in this report, it became apparent that lipid material isolated from different fish mairixes displayed varying GPC elution characteristics. This was demonstrated by the appearence of large

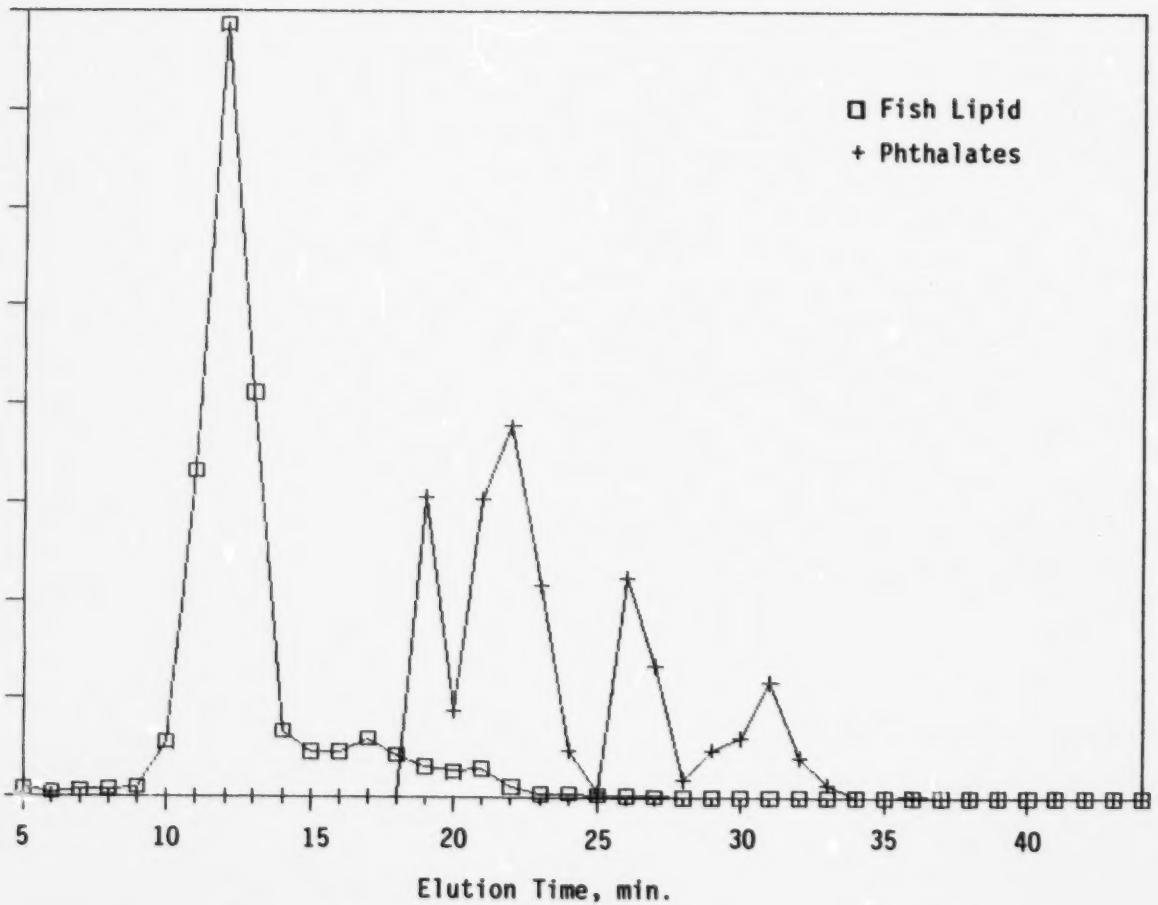


FIGURE 1. GPC ELUTION CHARACTERISTICS OF FISH LIPID AND PRIORITY POLLUTANT PHTHALATES

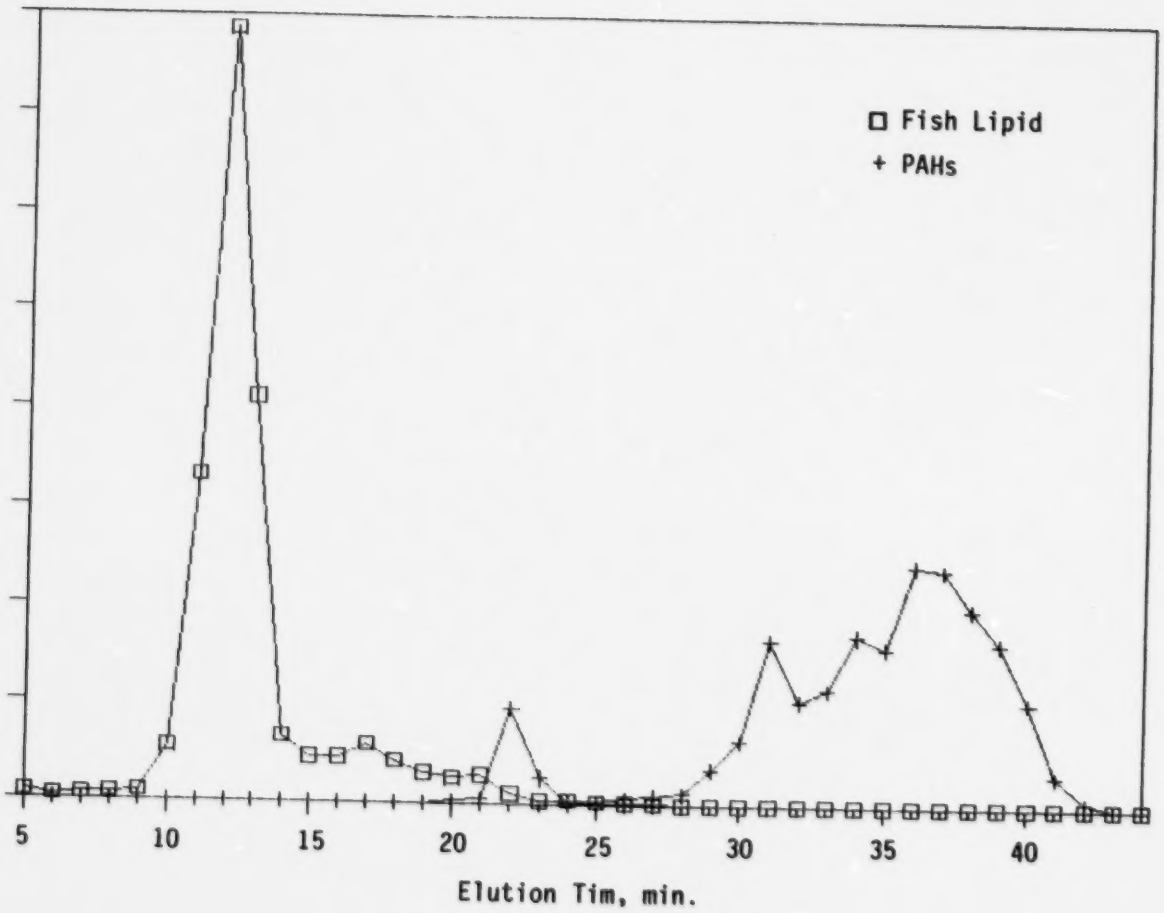


FIGURE 2. GPC ELUTION CHARACTERISTICS OF FISH LIPID AND PRIORITY POLLUTANT PAHs

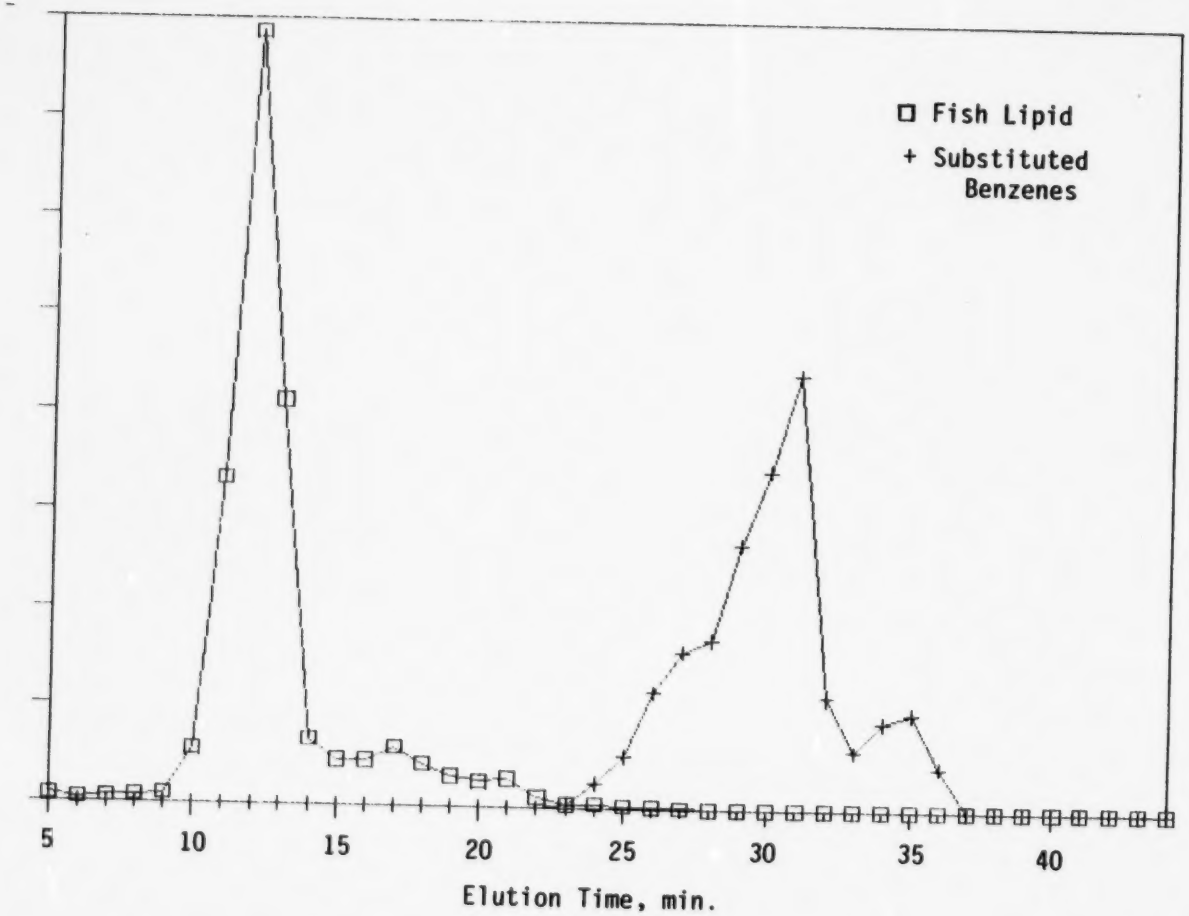


FIGURE 3. GPC ELUTION CHARACTERISTICS OF FISH LIPID AND PRIORITY POLLUTANT SUBSTITUTED BENZENES

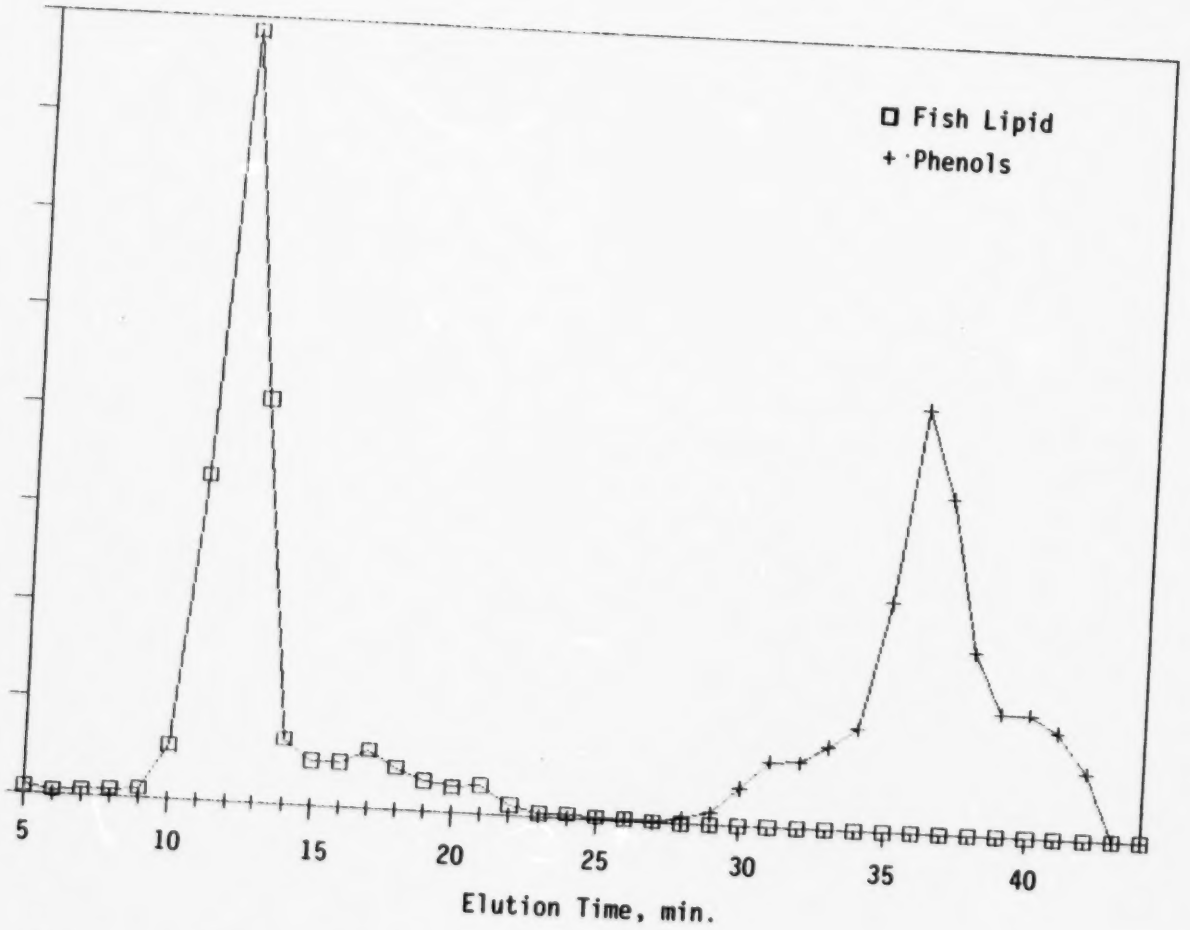


FIGURE 4. GPC ELUTION CHARACTERISTICS OF FISH LIPID AND PRIORITY POLLUTANT PHENOLS

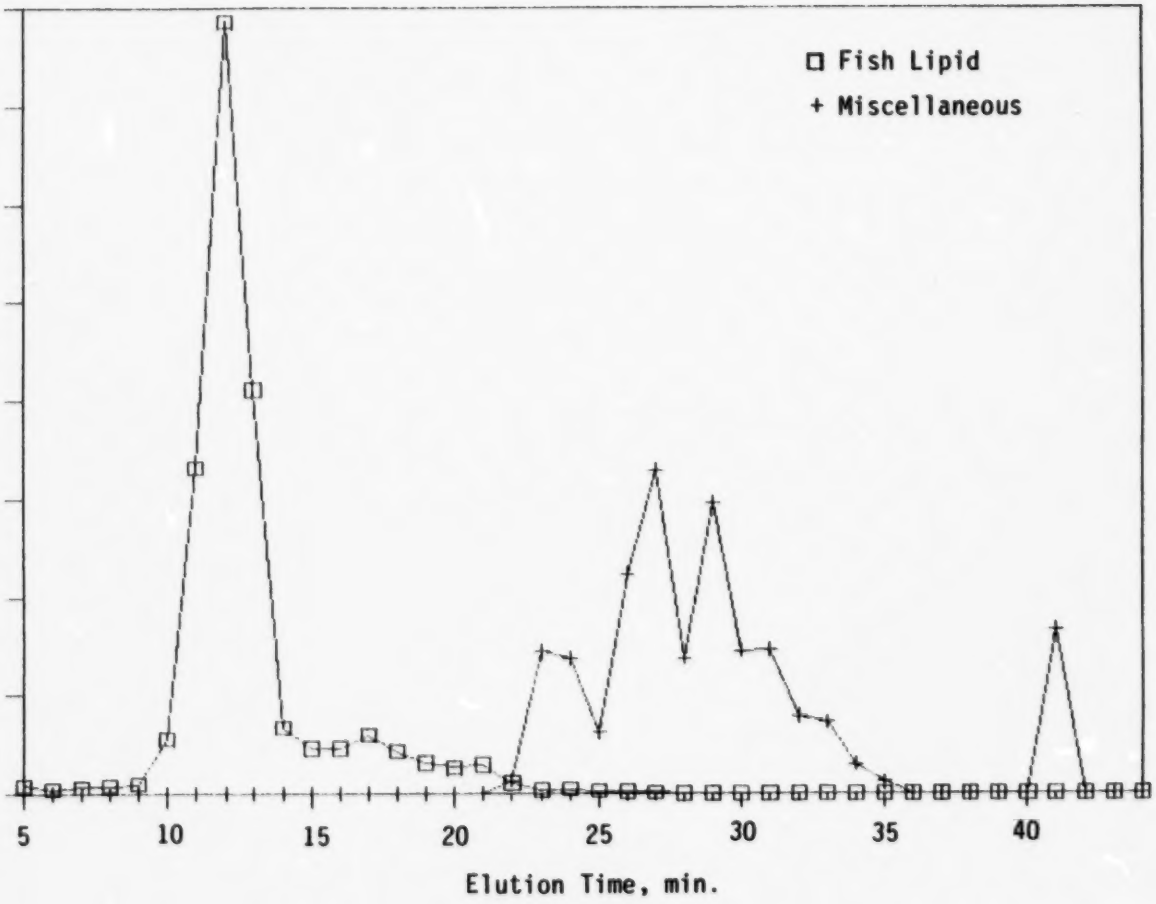


FIGURE 5. GPC ELUTION CHARACTERISTICS OF FISH LIPID AND PRIORITY POLLUTANT MISCELLANEOUS COMPOUNDS

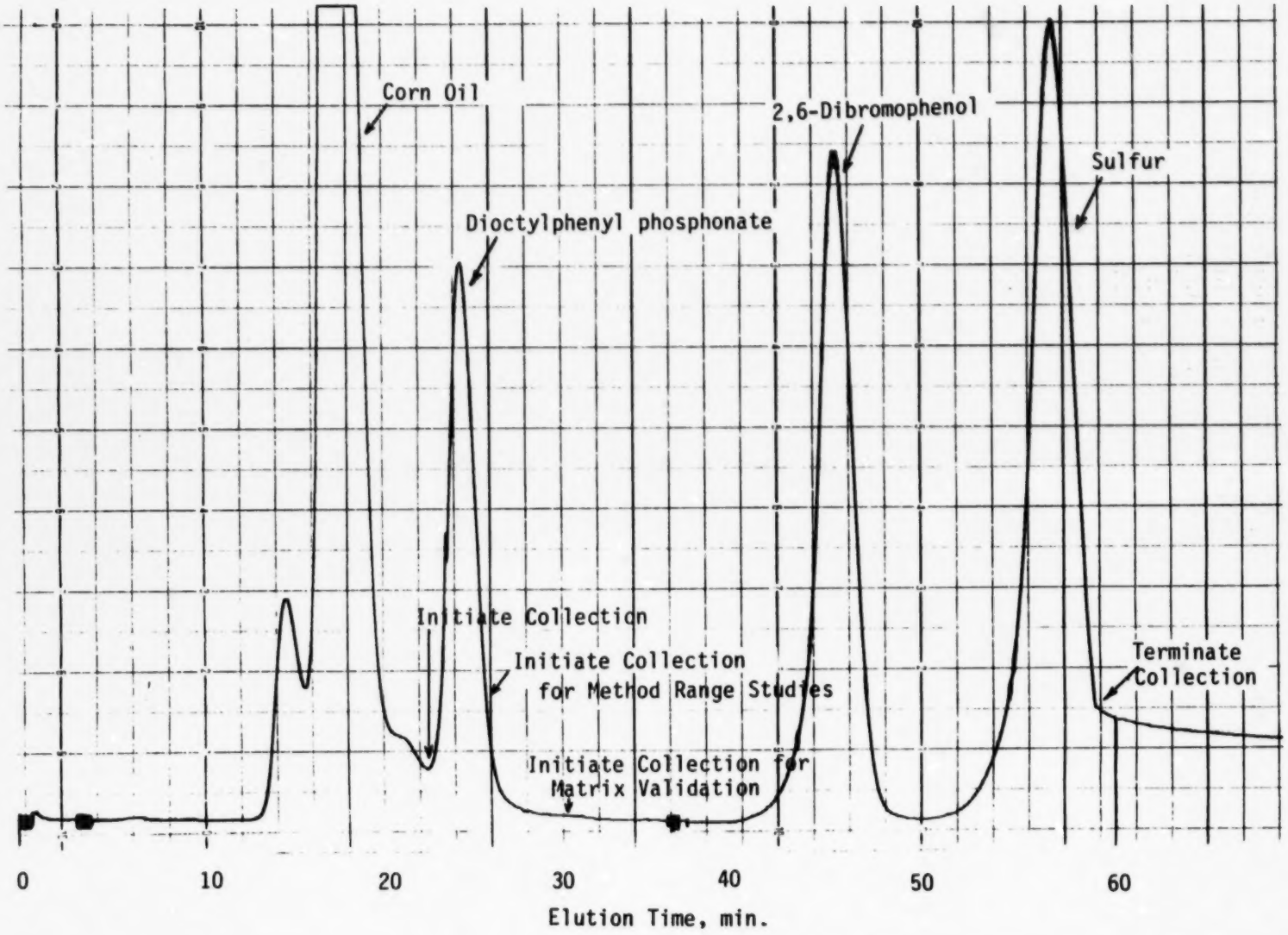


FIGURE 6. GPC-UV CHROMATOGRAM OF GPC CALIBRATION MIXTURE

late-eluting peaks in the GPC-UV chromatogram and by increased residue weight measurements generated from the collected GPC fraction. During the method validation studies when a mixture of scallops, catfish fillets, and salmon fillets were used as the fish matrix, sample collection was initiated when the dioctylphenyl phosphonate peak returned to 20 percent of baseline. When matrix validation studies were conducted using minnows as the fish matrix, it became necessary to delay sample collection four additional minutes. Sample collection initiation times used throughout the work assignment are indicated in Figure 6.

INSTRUMENT RANGE STUDIES AND PRELIMINARY METHOD EVALUATION

Instrument Range Studies

IDLs and estimated MDLs are given in Table 14. IDLs were generally equal to or less than 1 µg/mL with the exception of several of the phenols and pesticides. 2,4-Dinitrophenol and 2-methyl-4,6-dinitrophenol yielded IDLs greater than 1 µg/mL because of their chromatographic characteristics. Pesticides such as alpha- and gamma-chlordane, endosulfan sulfate, endosulfan I, endosulfan II, endrin, endrin aldehyde, and an octachlorobiphenyl yielded IDLs near or greater than 5 µg/mL because their mass spectra do not have intense characteristic ions. Benzidine and 3,3´-dichlorobenzidine were not detected presumably because of their chromatographic characteristics or because they were not stable in the solutions used for IDL determinations.

The IDL data were used to estimate the method detection limits that could be expected using the fish analysis method. A concentration factor of 10 is achieved using the fish analysis method; the extract resulting from 10 grams of fish is concentrated to 1 mL. Calculated method detection limits of the priority pollutant compounds in fish based on the IDL data are given in Table 15. Most of the priority pollutant compounds were calculated to be in the 10 to 100 ng/g concentration range.

Calibration data generated from analyses of solutions containing the priority pollutant compounds and recovery standards at the 0.1-100 µg/mL concentration ranges were tabulated; these data have been

TABLE 15. INSTRUMENT DETECTION LIMITS AND ESTIMATED METHOD DETECTION LIMITS

COMPOUND	IDL, UG/ML	ESTIMATED MDL, UG/G	COMPOUND	IDL, UG/ML	ESTIMATED MDL, UG/G
1,2,4-TRICHLOROBENZENE	0.5	0.05	D10-ACENAPHTHENE	0.4	0.04
1,2-DICHLOROBENZENE	0.4	0.04	D10-FLUORENE	0.4	0.04
1,3-DICHLOROBENZENE	0.4	0.04	D10-PYRENE	0.3	0.03
1,4-DICHLOROBENZENE	0.3	0.03	D3-2,4-DICHLOROPHENOL	0.8	0.08
2,4,6-TRICHLOROPHENOL	0.9	0.09	DDD	0.9	0.09
2,4-DICHLOROPHENOL	0.7	0.07	DDE	1.0	0.10
2,4-DIMETHYLPHENOL	0.8	0.08	DDT	1.0	0.10
2,4-DINITROPHENOL	7.0	0.70	DIBENZO(A,H)ANTHRACENE	0.9	0.09
2,4-DINITROTOLUENE	1.0	0.10	DIELDRIN	0.8	0.08
2,6-DINITROTOLUENE	1.0	0.10	DIETHYL PHTHALATE	0.1	0.01
2-CHLORONAPHTHALENE	0.3	0.03	DIMETHYL PHTHALATE	0.3	0.03
2-CHLOROPHENOL	0.5	0.05	DI-N-BUTYL PHTHALATE	0.1	0.01
2-METHYL-4,6-DINITROPHENOL	5.0	0.50	DI-N-OCTYL PHTHALATE	0.4	0.04
2-NITROPHENOL	0.9	0.09	ENDOSULFAN I	9.0	0.90
4-BROMOPHENYL PHENYLETHER	1.0	0.10	ENDOSULFAN II	6.0	0.60
4-CHLOROPHENYL PHENYLETHER	0.7	0.07	ENDOSULFAN SULFATE	6.0	0.60
4-CHLORO-3-METHYLPHENOL	0.6	0.06	ENDRIN	7.0	0.70
4-NITROPHENOL	1.0	0.10	ENDRIN ALDEHYDE	19.0	1.90
ACENAPHTHENE	0.3	0.03	FLUORANTHENE	0.3	0.03
ACENAPHTHYLENE	0.2	0.02	FLUORENE	0.1	0.01
ALDRIN	0.7	0.07	HEPTACHLOR	1.0	0.10
ANTHRACENE	0.3	0.03	HEPTACHLOR EPOXIDE	1.0	0.10
BENZO(A)ANTHRACENE	0.6	0.06	HEXACHLOROBENZENE	0.9	0.09
BENZO(A)PYRENE	0.5	0.05	HEXACHLOROBUTADIENE	0.8	0.08
BENZO(B&K)FLUORANTHENE	1.0	0.10	HEXACHLOROCYCLOPENTADIENE	0.9	0.09
BENZO(G,H,I)PERYLENE	0.9	0.09	HEXACHLOROETHANE	0.7	0.07
BHC(ALPHA)	1.0	0.10	INDENO(1,2,3-CD)PYRENE	0.8	0.08
BHC(BETA)	2.0	0.20	ISOPHORONE	0.3	0.03
BHC(DELTA)	1.0	0.10	NAPHTHALENE	0.1	0.01
BHC(GAMMA)	1.0	0.10	NITROBENZENE	1.0	0.10
BIS-(2-CHLOROETHOXY) METHANE	0.3	0.03	N-NITROSODIPHENYLAMINE	0.6	0.06
BIS-(2-CHLOROETHYL) ETHER	0.4	0.04	N-NITROSODIPROPYLAMINE	1.0	0.10
BIS-(2-CHLOROISOPROPYL) ETHER	0.8	0.08	OCTACHLOROBIPHENYL	7.0	0.70
BIS-(2-ETHYLHEXYL) PHTHALATE	0.4	0.04	PENTACHLOROPHENOL	2.0	0.20
BUTYLBENZYL PHTHALATE	0.6	0.06	PHENANTHRENE	0.04	0.004
CHLORDANE(ALPHA)	5.0	0.50	PHENOL	0.3	0.03
CHLORDANE(GAMMA)	5.0	0.50	PYRENE	0.3	0.03
CHRYSENE	0.4	0.04	TRICHLOROBIPHENYL	1.0	0.10

statistically evaluated to determine the calibration characteristics of each of the compounds.

Preliminary Method Evaluation

Recovery data from the seven spiked and one unspiked preliminary method evaluation fish samples as determined by GC-MS are given in Table 16. An example of the GC-MS chromatogram generated during analysis of one of the spiked method evaluation fish samples is shown in Figure 7. Most of the priority pollutant compounds demonstrated recoveries greater than 70 percent. These recoveries are generally 10 to 15 percent lower than those obtained when the compounds were spiked into the fish after the homogenization in acetonitrile as reported in Table 14. The lower recoveries may be due to extraction losses during the acetonitrile homogenization step, degradation of the compounds by components of the fish tissue, or matrix effects due to the use of different fish tissue. Significantly lower recoveries were observed for the more polar phenols; recoveries of 20 percent, 53 percent and 28 percent were obtained for 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, and 4-nitrophenol, respectively. Possibly these components were incorporated into the fish tissue and degraded by or inefficiently removed from the tissue. High recoveries, greater than 150 percent, were observed for bis-(2-ethylhexyl) phthalate and butylbenzyl phthalate. These phthalates are common contaminants introduced during sample processing.

The percent relative standard deviations (%RSD) generated from the seven spiked samples were generally around 20 percent. The high %RSD values generally do not reflect variability in compound recoveries; in most cases equally high %RSD values were obtained for the GC-MS response factors generated during analysis of the standard solution as demonstrated in Table 17. These response factors were calculated using internal standard techniques with d_{10}-phenanthrene as the internal standard. Data from the four files were generated at one concentration level.

Recoveries of priority pollutant compounds from spiked fish samples that were stored for 24 hours prior to processing are given in Table 18. Recovery data were not obtained for dieldrin; an interference was present preventing quantification of dieldrin. The results obtained

TABLE 16. PRELIMINARY METHOD EVALUATION RESULTS

COMPOUND	SPIKE LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE 1	2	3	4	5	6	7	AVG	%RSD
1,2,4-TRICHLOROBENZENE	2	59	81	89	69	84	80	78	77	12
1,2-DICHLOROBENZENE	2	59	89	99	67	92	84	71	80	17
1,3-DICHLOROBENZENE	2	51	71	89	57	77	73	50	67	20
1,4-DICHLOROBENZENE	2	53	80	91	63	89	80	65	74	18
2,4,6-TRICHLOROPHENOL	2	74	61	63	93	73	93	100	79	18
2,4-DICHLOROPHENOL	2	75	68	72	89	88	94	95	83	12
2,4-DIMETHYLPHENOL	2	69	69	72	100	79	92	92	82	14
2,4-DINITROPHENOL	12	16	16	17	15	19	23	24	19	18
2,4-DINITROTOLUENE	2	54	69	75	69	74	69	74	69	10
2,6-DINITROTOLUENE	2	57	69	74	71	78	75	78	72	10
2-CHLORONAPHTHALENE	2	59	74	81	73	83	79	80	75	10
2-CHLOROPHENOL	2	58	61	71	65	72	81	67	68	10
2-METHYL-4,6-DINITROPHENOL	12	39	40	49	44	52	61	62	50	17
2-NITROPHENOL	2	67	59	67	78	80	91	80	75	13
4,4'-DDD	2	81	114	126	72	104	108	106	102	17
4,4'-DDE	2	82	130	115	74	93	93	107	99	18
4,4'-DDT	2	68	93	104	49	84	91	78	81	21
4-BROMOPHENYL PHENYL ETHER	2	60	74	80	82	81	78	92	78	11
4-CHLOROPHENYL PHENYL ETHER	2	61	76	82	80	84	79	87	79	10
4-CHLORO-3-METHYLPHENOL	2	78	66	70	102	85	97	105	86	17
4-NITROPHENOL	2	11	(C)	(C)	(C)	29	18	47	26	52
ACENAPHTHENE	2	57	63	63	81	76	70	82	70	13
ACENAPHTHYLENE	2	59	71	75	79	83	74	84	75	11
ALDRIN	2	47	81	105	71	88	68	58	74	24
ALPHA-BHC	2	56	73	83	70	77	75	80	74	11
ALPHA-CHLORDANE	12	67	81	89	62	68	85	93	78	14
ANTHRACENE	2	56	67	64	85	76	73	90	73	15
BENZO(A)ANTHRACENE	2	67	95	104	54	88	85	83	82	19
BENZO(A)PYRENE	2	89	116	133	60	105	109	95	101	21
BENZO(B &/OR K)FLUORANTHENE	2	68	(C)	109	50	91	86	91	83	23
BENZO(G,H,I,)PERYLENE	2	86	62	61	28	45	78	102	66	35
BETA-BHC	2	54	73	78	61	71	81	85	72	14
BIS(2-CHLOROETHYL) ETHER	2	41	69	84	48	76	64	51	62	23
BIS-2-CHLOROETHOXYMETHANE	2	52	79	86	65	93	76	63	73	18
BIS-(2-CHLOROISOPROPYL) ETHER	2	61	89	107	80	128	66	52	83	30
BIS-(2-ETHYLHEXYL) PHTHALATE	2	147	216	252	117	205	209	171	188	23
BUTYLBENZYL PHTHALATE	2	129	175	196	136	164	167	147	159	14
CHRYSENE	2	67	95	104	54	88	85	83	82	19
DELTA-BHC	2	55	72	85	59	78	76	73	71	14
DIBENZO(A,H)ANTHRACENE	2	99	131	142	65	99	124	100	109	22
DIELDRIN	2	48	100	92	58	83	(C)	55	73	27
DIETHYL PHTHALATE	2	55	69	74	88	79	75	82	75	13
DIMETHYL PHTHALATE	2	47	56	59	67	64	61	71	61	12
DI-N-BUTYL PHTHALATE	2	84	105	113	104	113	108	108	105	9
DI-N-OCTYL PHTHALATE	2	119	172	200	85	159	(C)	127	123	50
ENDOSULFAN SULFATE	10	69	95	98	52	81	90	77	80	19
ENDOSULFAN I	20	59	83	91	60	73	76	75	74	14
ENDOSULFAN II	10	66	94	102	60	85	81	79	81	17
ENDRIN	10	94	149	160	89	138	127	111	124	20
ENDRIN ALDEHYDE	40	12	38	45	25	43	23	10	28	47
FLUORANTHENE	2	65	91	97	66	85	85	85	82	13
FLUORENE	2	59	76	81	79	84	77	85	77	10
GAMMA-BHC	2	52	88	97	73	92	73	78	79	18
GAMMA-CHLORDANE	10	66	78	86	57	66	83	90	75	15
HEPTACHLOR	2	53	76	82	69	80	73	71	72	12
HEPTACHLOR EPOXIDE	2	63	97	109	70	104	87	74	86	19
HEXACHLOROBENZENE	2	54	68	77	70	67	70	92	71	15
HEXACHLOROBUTADIENE	2	58	74	79	69	73	75	81	73	10
HEXACHLOROCYCLOPENTADIENE	2	19	26	25	18	25	28	7	21	32
HEXACHLOROETHANE	2	59	87	97	68	92	84	70	79	17
INDENO(1,2,3-CD)PYRENE	2	126	161	174	82	125	156	129	136	21
ISOPHORONE	2	39	64	68	55	71	55	51	58	18
NAPHTHALENE	2	68	97	106	82	106	(D)	85	91	15
NITROBENZENE	2	53	78	88	63	84	77	64	73	16
N-NITROSODIMETHYLAMINE	2	43	78	104	54	87	63	50	68	30
N-NITROSODIPHENYLAMINE	2	55	46	49	122	56	57	98	69	39
N-NITROSODIPROPYLAMINE	2	47	73	83	69	96	55	51	68	24
PENTACHLOROPHENOL	2	91	80	85	103	91	118	133	100	18
PHENANTHRENE	2	59	82	91	73	84	76	82	78	12
PHENOL	2	31	45	49	38	45	50	47	43	14
PYRENE	2	65	89	97	64	86	81	82	81	14
TRICHLOROBIPHENYL	2	56	75	84	71	70	74	85	74	12
D10-ACENAPHTHENE (B)	2	60	60	59	85	83	72	91	73	17
D10-FLUORENE (B)	2	62	73	75	81	89	78	90	78	11
D10-PYRENE (B)	2	73	93	100	71	98	95	99	90	13
D3-2,4-DICHLOROPHENOL (B)	2	63	67	70	75	75	81	80	73	8

(A) CORRECTED FOR AMOUNT FOUND IN BLANK.
(B) RECOVERY STANDARD ADDED TO EACH SAMPLE PRIOR TO EXTRACTION TO OBTAIN AN INDICATION OF METHOD PERFORMANCE.
(C) COMPOUND NOT DETECTED.
(D) PEAK SATURATED DETECTOR.

TABLE 17. GC-MS RESPONSE FACTORS GENERATED DURING PRELIMINARY METHOD EVALUATION

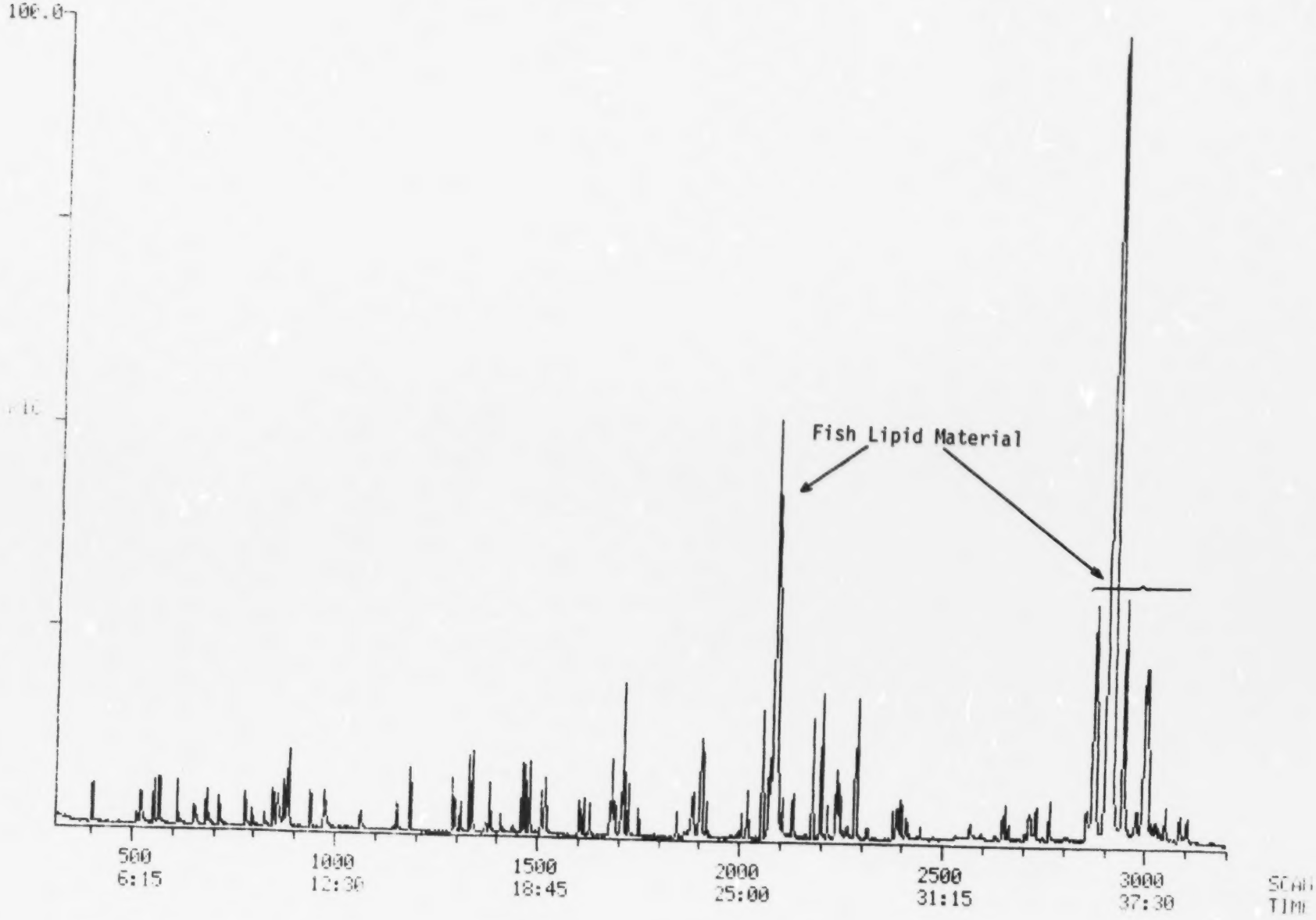
COMPOUND	RESPONSE FACTORS IN GIVEN FILE 1	2	3	4	AVG	%RSD
1,2,4-TRICHLOROBENZENE	0.393	0.384	0.466	0.325	0.392	13
1,2-DICHLOROBENZENE	0.529	0.539	0.677	0.433	0.544	16
1,3-DICHLOROBENZENE	0.568	0.383	0.540	0.323	0.453	23
1,4-DICHLOROBENZENE	0.568	0.512	0.714	0.494	0.572	15
2,4,6-TRICHLOROPHENOL	0.155	0.142	0.142	0.137	0.144	5
2,4-DICHLOROPHENOL	0.260	0.267	0.266	0.223	0.254	7
2,4-DIMETHYLPHENOL	0.200	0.207	0.173	0.173	0.188	8
2,4-DINITROPHENOL	0.069	0.053	0.068	0.049	0.060	15
2,4-DINITROTOLUENE	0.236	0.243	0.247	0.218	0.236	5
2,6-DINITROTOLUENE	0.201	0.189	0.214	0.164	0.192	10
2-CHLORONAPHTHALENE	0.783	0.767	0.869	0.649	0.767	10
2-CHLOROPHENOL	0.341	0.290	0.356	0.292	0.320	9
2-METHYL-4,6-DINITROPHENOL	0.088	0.075	0.087	0.069	0.080	10
2-NITROPHENOL	0.195	0.175	0.207	0.151	0.182	12
4,4'-DDD	0.195	0.196	0.224	0.226	0.210	7
4,4'-DDE	0.145	0.139	0.165	0.145	0.149	6
4,4'-DDT	0.266	0.234	0.334	0.216	0.262	17
4-BROMOPHENYL PHENYL ETHER	0.153	0.144	0.153	0.146	0.149	3
4-CHLOROPHENYL PHENYL ETHER	0.307	0.288	0.319	0.278	0.298	5
4-CHLORO-3-METHYLPHENOL	0.214	0.198	0.183	0.174	0.192	8
4-NITROPHENOL	0.047	0.056	0.034	0.045	0.046	17
ACENAPHTHENE	0.675	0.699	0.625	0.614	0.653	5
ACENAPHTHYLENE	(C)	1.155	1.087	0.989	1.077	6
ALDRIN	0.191	0.235	0.250	0.196	0.218	12
ALPHA-BHC	0.126	0.133	0.145	0.122	0.132	7
ALPHA-CHLORDANE	0.050	0.035	0.056	0.042	0.046	17
ANTHRACENE	0.736	0.902	0.627	0.855	0.780	14
BENZO(A)ANTHRACENE	0.514	0.509	0.555	0.496	0.519	4
BENZO(A)PYRENE	0.313	0.270	0.268	0.356	0.302	12
BENZO(B &/OR K)FLUORANTHENE	0.400	0.347	0.379	0.450	0.394	9
BENZO(G,H,I)PERYLENE	0.247	0.242	0.186	0.293	0.242	16
BETA-BHC	0.072	0.067	0.079	0.071	0.072	6
BIS(2-CHLOROETHYL) ETHER	0.578	0.480	0.720	0.478	0.564	17
BIS-2-CHLOROETHOXYMETHANE	0.672	0.709	0.853	0.584	0.705	14
BIS-(2-CHLOROISOPROPYL) ETHER	0.562	1.072	1.221	0.726	0.895	29
BIS-(2-ETHYLHEXYL) PHTHALATE	0.311	0.310	0.370	0.496	0.372	20
BUTYLBENZYL PHTHALATE	0.248	0.234	0.283	0.339	0.276	15
CHRYSENE	0.514	0.509	0.555	0.496	0.519	4
DELTA-BHC	0.093	0.094	0.110	0.093	0.098	8
DIBENZO(A,H)ANTHRACENE	0.271	0.250	0.227	0.313	0.265	12
DIELDRIN	0.245	0.303	0.323	0.217	0.272	16
DIETHYL PHTHALATE	0.621	0.664	0.671	0.650	0.652	3
DIMETHYL PHTHALATE	0.680	0.702	0.683	0.639	0.676	3
DI-N-BUTYL PHTHALATE	0.777	0.844	0.896	1.019	0.884	10
DI-N-OCTYL PHTHALATE	0.514	0.525	0.615	0.834	0.622	21
ENDOSULFAN SULFATE	0.048	0.040	0.060	0.040	0.047	18
ENDOSULFAN I	0.032	0.029	0.037	0.026	0.031	13
ENDOSULFAN II	0.065	0.059	0.082	0.051	0.064	17
ENDRIN	0.026	0.032	0.033	0.031	0.031	9
ENDRIN ALDEHYDE	0.079	0.099	0.112	0.066	0.089	20
FLUORANTHENE	0.784	0.851	0.754	0.790	0.795	4
FLUORENE	0.785	0.768	0.787	0.675	0.754	6
GAMMA-BHC	0.123	0.135	0.146	0.110	0.129	10
GAMMA-CHLORDANE	0.047	0.032	0.053	0.035	0.042	20
HEPTACHLOR	0.183	0.205	0.230	0.185	0.201	9
HEPTACHLOR EPOXIDE	0.103	0.135	0.139	0.112	0.122	12
HEXACHLOROBENZENE	0.175	0.158	0.174	0.167	0.168	4
HEXACHLOROBUTADIENE	0.175	0.174	0.193	0.156	0.174	8
HEXACHLOROCYCLOPENTADIENE	0.124	0.113	0.127	0.101	0.116	9
HEXACHLOROETHANE	0.262	0.263	0.329	0.208	0.266	16
INDENO(1,2,3-CD)PYRENE	0.252	0.226	0.173	0.385	0.259	30
ISOPHORONE	0.897	0.943	1.082	0.841	0.940	9
NAPHTHALENE	(C)	1.486	(C)	1.147	1.316	13
NITROBENZENE	0.285	0.279	0.348	0.226	0.284	15
N-NITROSODIMETHYLAMINE	0.084	0.060	0.092	0.065	0.075	18
N-NITROSODIPHENYLAMINE	0.197	0.332	0.129	0.236	0.224	33
N-NITROSODIPROPYLAMINE	0.130	0.170	0.186	0.145	0.158	14
PENTACHLOROPHENOL	0.065	0.058	0.062	0.064	0.062	4
PHENANTHRENE	0.997	1.022	1.068	0.882	0.992	7
PHENOL	0.404	0.321	0.423	0.301	0.362	14
PYRENE	0.776	0.872	0.754	0.813	0.804	6
TRICHLOROBIPHENYL	0.172	0.160	0.182	0.158	0.168	6
D10-ACENAPHTHENE (A)	0.525	0.552	0.472	0.509	0.514	6
D10-FLUORENE (A)	0.551	0.550	0.553	0.517	0.543	3
D10-PYRENE (A)	0.550	0.669	0.507	0.712	0.610	14
D3-2,4-DICHLOROPHENOL (A)	0.297	0.306	0.323	0.266	0.298	7

(A) RECOVERY STANDARD.
(B) COMPOUND NOT DETECTED.
(C) PEAK SATURATED DETECTOR.

TABLE 18. 24-HOUR STUDY RESULTS

COMPOUND	SPIKE LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE 1	2	3	4	5	6	7	AMOUNT FOUND IN BLANK, UG/G	AVG(A)	%RSD
1,2,4-TRICHLOROBENZENE	2	62	55	58	64	52	28	53	(C)	53	21
1,2-DICHLOROBENZENE	2	40	51	57	65	54	28	36	(C)	47	25
1,3-DICHLOROBENZENE	2	42	55	47	54	57	37	35	(C)	47	18
1,4-DICHLOROBENZENE	2	49	60	55	62	55	33	49	(C)	52	18
2,4,6-TRICHLOROPHENOL	2	90	90	90	104	76	46	80	(C)	82	21
2,4-DICHLOROPHENOL	2	83	74	85	91	71	40	69	(C)	73	21
2,4-DIMETHYLPHENOL	2	100	92	98	110	87	44	81	(C)	87	23
2,4-DINITROPHENOL	12	21	20	18	27	14	11	18	(C)	18	26
2,4-DINITROTOLUENE	2	39	33	37	40	34	18	33	(C)	33	20
2,6-DINITROTOLUENE	2	41	39	41	48	38	21	36	(C)	38	20
2-CHLORONAPHTHALENE	2	49	45	48	55	47	27	44	(C)	45	18
2-CHLOROPHENOL	2	53	60	72	88	78	52	66	(C)	70	18
2-METHYL-4,6-DINITROPHENOL	12	56	47	43	53	33	24	38	(C)	42	25
2-NITROPHENOL	2	74	69	75	83	63	33	71	(C)	67	22
4,4'-DDD	2	105	104	92	105	47	50	83	(C)	84	28
4,4'-DDE	2	315	350	491	437	239	295	408	(C)	362	22
4,4'-DDT	2	185	136	92	193	86	106	175	(C)	139	30
4-BROMOPHENYL PHENYL ETHER	2	85	81	80	93	69	51	74	(C)	76	16
4-CHLOROPHENYL PHENYL ETHER	2	66	60	63	73	60	35	60	(C)	60	18
4-CHLORO-3-METHYLPHENOL	2	83	75	87	99	64	33	74	(C)	74	26
4-NITROPHENOL	2	11	(C)	13	15	24	8	10	(C)	12	58
ACENAPHTHENE	2	50	48	45	54	46	23	42	(C)	44	21
ACENAPHTHYLENE	2	48	45	47	52	35	19	41	(C)	41	25
ALDRIN	2	94	83	91	96	51	47	87	(C)	78	24
ALPHA-BHC	2	54	50	51	62	37	26	46	(C)	47	23
ALPHA-CHLORDANE	2	230	204	169	216	129	163	186	(C)	188	16
ANTHRACENE	2	59	59	59	64	45	31	54	(C)	53	20
BENZO(A)ANTHRACENE	2	72	70	67	65	60	36	50	(C)	63	22
BENZO(A)PYRENE	2	79	81	70	77	49	40	68	(C)	66	23
BENZO(B &/OR K)FLUORANTHENE	2	71	169	129	173	85	134	129	(C)	127	28
BENZO(G,H,I,)PERYLENE	2	50	78	75	92	39	60	88	(C)	66	25
BETA-BHC	2	149	88	79	90	162	84	74	(C)	104	32
BIS(2-CHLOROETHYL) ETHER	2	37	45	52	54	56	26	41	(C)	44	22
BIS-2-CHLOROETHOXYMETHANE	2	42	39	44	50	34	18	41	(C)	38	25
BIS-(2-CHLOROISOPROPYL) ETHER	2	48	45	61	67	42	25	47	(C)	48	26
BIS-(2-ETHYLHEXYL) PHTHALATE	2	111	103	96	113	160	52	89	(C)	103	29
BUTYLBENZYL PHTHALATE	2	294	201	140	363	(D)	139	308	(C)	441	113
CHRYSENE	2	74	69	67	88	80	44	64	(C)	70	19
DELTA-BHC	2	50	53	52	58	36	27	44	(C)	46	22
DIBENZO(A,H)ANTHRACENE	2	90	122	90	114	45	82	157	(C)	97	36
DIELDRIN	2	(D)	(D)	(D)	(D)	(D)	(D)	(D)	(C)	-	-
DIETHYL PHTHALATE	2	61	59	59	72	155	41	52	(C)	71	50
DIMETHYL PHTHALATE	2	45	42	43	53	55	22	40	(C)	43	23
DI-N-BUTYL PHTHALATE	2	107	96	91	117	117	56	90	(C)	96	20
DI-N-OCTYL PHTHALATE	2	78	75	69	85	52	38	66	(C)	66	22
ENDOSULFAN SULFATE	10	184	147	114	193	84	109	186	(C)	143	32
ENDOSULFAN I	20	165	140	153	194	104	105	159	(C)	146	21
ENDOSULFAN II	10	51	45	44	60	22	29	47	(C)	43	28
ENDRIN	10	148	138	137	172	168	77	160	(C)	143	21
ENDRIN ALDEHYDE	40	15	10	13	19	(C)	9	10	(C)	11	48
FLUORANTHENE	2	60	56	53	63	47	31	48	(C)	51	19
FLUORENE	2	47	45	45	53	44	23	42	(C)	43	20
GAMMA-BHC	2	100	94	55	73	85	48	56	(C)	73	26
GAMMA-CHLORDANE	10	250	228	206	249	141	223	213	(C)	216	16
HEPTACHLOR	2	38	37	37	43	21	16	34	(C)	32	28
HEPTACHLOR EPOXIDE	2	54	48	74	62	45	33	52	(C)	52	23
HEXACHLOROBENZENE	2	97	93	87	97	70	63	81	(C)	84	15
HEXACHLOROBUTADIENE	2	78	75	77	84	60	42	69	(C)	69	19
HEXACHLOROCYCLOPENTADIENE	2	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	-	-
HEXACHLOROETHANE	2	48	46	50	59	38	23	40	(C)	43	24
INDENO(1,2,3-CD)PYRENE	2	114	142	111	146	48	74	205	(C)	120	39
ISOPHORONE	2	46	44	51	62	38	23	47	(C)	44	25
NAPHTHALENE	2	56	51	55	62	51	27	53	(C)	51	20
NITROBENZENE	2	42	44	45	54	43	22	33	(C)	40	23
N-NITROSODIMETHYLAMINE	2	9	100	(C)	129	97	55	(C)	(C)	56	89
N-NITROSODIPHENYLAMINE	2	109	95	102	114	128	46	96	(C)	99	24
N-NITROSODIPROPYLAMINE	2	29	81	87	76	49	25	60	(C)	52	34
PENTACHLOROPHENOL	2	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	-	-
PHENANTHRENE	2	69	46	59	73	86	35	59	(C)	61	26
PHENOL	2	31	40	39	49	47	23	42	(C)	39	21
PYRENE	2	64	61	54	66	37	41	50	(C)	53	20
TRICHLOROBIPHENYL	2	140	125	122	143	114	87	126	(C)	123	14
D10-ACENAPHTHENE (B)	2	67	69	72	73	57	34	47	-	60	23
D10-FLUORENE (B)	2	62	66	70	67	47	31	44	-	55	25
D10-PYRENE (B)	2	86	90	93	88	57	48	60	-	75	23
D3-2,4-DICHLOROPHENOL (B)	2	75	72	83	77	66	36	57	-	67	22

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD ADDED TO EACH SAMPLE PRIOR TO EXTRACTION TO OBTAIN AN INDICATION OF METHOD PERFORMANCE.
(C) THE COMPOUND WAS NOT DETECTED.
(D) THE PEAK SATURATED THE DETECTOR.



Scan Number/Retention Time, min.

FIGURE 7. GC-MS CHROMATOGRAM OF SPIKED PRELIMINARY METHOD EVALUATION FISH SAMPLE

for alpha- and gamma-chlordane, endosulfan sulfate, endosulfan I, endrin, and trichlorobiphenyl are in doubt; the integrity of the solution used to add these compounds to the fish is questionable. Phthalates are frequently introduced during sample processing; high recoveries for bis-(2-ethylhexyl) phthalate and butylbenzyl phthalate may reflect phthalate contamination of the samples during sample processing. High recoveries of 4,4'-DDE and 4,4'-DDT are due to the presence of interferences in the fish extracts. Two compounds, hexachlorocyclopentadiene and pentachlorophenol, were not recovered from the fish extracts. Hexachlorocyclopentadiene poses analytical problems because of its tendency to decompose in a hot injection port; coextracted components may accelerate the decomposition. Low recoveries of this compound do not necessarily reflect removal difficulties from the fish matrix. Pentachlorophenol was recovered from the fish matrix with 97 percent efficiency during the preliminary method evaluation study. Failure to detect this compound in 24-hour storage study samples may indicate incorporation of this material into the fish matrix or decomposition of the pentachlorophenol during the 24-hour storage period.

Recovery and percent relative standard deviation data for the preliminary method evaluation and 24-hour storage studies are compared in Table 19. As observed during the preliminary method evaluation, percent relative standard deviations calculated for the seven samples were generally 15 to 30 percent. Considering these statistical data, significant decreases in recoveries upon storage were observed for only pentachlorophenol, bis-(2-ethylhexyl) phthalate, and hexachlorocyclopentadiene.

A general trend of slightly lower recoveries is observed in samples that have been stored for 24 hours after spiking. This trend may be due to enzymatic degradation of the compounds or to incomplete extraction of compounds that became incorporated into the fish tissue. The experimental design does not allow for any conclusions regarding these data, but these data do suggest that recoveries observed immediately after spiking may be slightly overstated.

Most of the priority pollutant compounds were detected in the unspiked preliminary method evaluation fish sample at low levels. Recovery data for the spiked fish samples given in Table 16 have not been corrected for the amounts of compounds detected in the unspiked fish sample. It is

TABLE 19. COMPARISON OF PRELIMINARY METHOD EVALUATION AND 24-HOUR STORAGE STUDY RESULTS

COMPOUND	SPIKE LEVEL, UG/G	PRELIMINARY EVALUATION AVG (A)	PRELIMINARY EVALUATION %RSD	24-HOUR STUDY AVG(A)	24-HOUR STUDY %RSD	RECOVERY DIFFERENCE (E)
1,2,4-TRICHLOROBENZENE	2	77	12	53	21	18
1,2-DICHLOROBENZENE	2	80	17	47	25	27
1,3-DICHLOROBENZENE	2	67	20	47	18	16
1,4-DICHLOROBENZENE	2	74	18	52	18	18
2,4,6-TRICHLOROPHENOL	2	79	18	82	21	-10
2,4-DICHLOROPHENOL	2	83	12	73	21	2
2,4-DIMETHYLPHENOL	2	82	14	87	23	-12
2,4-DINITROPHENOL	12	19	18	18	26	0
2,4-DINITROTOLUENE	2	69	10	33	20	30
2,6-DINITROTOLUENE	2	72	10	38	20	28
2-CHLORONAPHTHALENE	2	75	10	45	18	24
2-CHLOROPHENOL	2	68	10	70	18	-7
2-METHYL-4,6-DINITROPHENOL	12	50	17	42	25	5
2-NITROPHENOL	2	75	13	67	22	1
4,4'-DDD	2	102	17	84	28	10
4,4'-DDE	2	99	18	362	22	-271
4,4'-DDT	2	81	21	139	30	-64
4-BROMOPHENYL PHENYL ETHER	2	78	11	76	16	-4
4-CHLOROPHENYL PHENYL ETHER	2	79	10	60	18	12
4-CHLORO-3-METHYLPHENOL	2	86	17	74	26	5
4-NITROPHENOL	2	26	52	13	38	8
ACENAPHTHENE	2	70	13	44	21	21
ACENAPHTHYLENE	2	75	11	41	25	28
ALDRIN	2	74	24	78	24	-8
ALPHA-BHC	2	74	11	47	23	21
ALPHA-CHLORDANE	12	78	14	188	16	-117
ANTHRACENE	2	73	15	52	20	16
BENZO(A)ANTHRACENE	2	82	19	63	22	13
BENZO(A)PYRENE	2	101	21	66	23	26
BENZO(B &/OR K)FLUORANTHENE	2	83	23	127	28	-61
BENZO(G,H,I,)PERYLENE	2	66	35	66	25	-6
BETA-BHC	2	72	14	104	32	-37
BIS(2-CHLOROETHYL) ETHER	2	62	23	44	22	15
BIS-2-CHLOROETHOXYMETHANE	2	73	18	38	25	31
BIS-(2-CHLORCISOPROPYL) ETHER	2	83	30	48	26	31
BIS-(2-ETHYLHEXYL) PHTHALATE	2	188	23	92	29	80
BUTYLBENZYL PHTHALATE	2	159	14	234	36	-89
CHRYSENE	2	82	19	70	19	6
DELTA-BHC	2	71	14	46	22	20
DIBENZO(A,H)ANTHRACENE	2	109	22	97	36	2
DIELDRIN	2	73	27	(D)	(D)	61
DIETHYL PHTHALATE	2	75	13	69	50	0
DIMETHYL PHTHALATE	2	61	12	43	23	14
DI-N-BUTYL PHTHALATE	2	105	9	96	20	-1
DI-N-OCTYL PHTHALATE	2	123	50	66	22	48
ENDOSULFAN SULFATE	10	80	19	143	32	-69
ENDOSULFAN I	20	74	14	146	21	-78
ENDOSULFAN II	10	81	17	43	28	32
ENDRIN	10	124	20	143	21	-28
ENDRIN ALDEHYDE	40	28	47	11	48	21
FLUORANTHENE	2	82	13	51	19	25
FLUORENE	2	77	10	43	20	28
GAMMA-BHC	2	79	18	73	26	2
GAMMA-CHLORDANE	10	75	15	216	16	-147
HEPTACHLOR	2	72	12	32	28	35
HEPTACHLOR EPOXIDE	2	86	19	52	23	28
HEXACHLOROBENZENE	2	71	15	84	15	-18
HEXACHLOROBUTADIENE	2	73	10	69	19	-3
HEXACHLOROCYCLOPENTADIENE	2	21	32	(C)	(C)	23
HEXACHLOROETHANE	2	79	17	43	24	31
INDENO(1,2,3-CD)PYRENE	2	136	21	120	39	3
ISOPHORONE	2	58	18	44	25	10
NAPHTHALENE	2	91	15	51	20	22
NITROBENZENE	2	73	16	40	23	27
N-NITROSODIMETHYLAMINE	2	68	30	78	53	-12
N-NITROSODIPHENYLAMINE(AS DIPH	2	69	39	99	24	-32
N-NITROSODIPROPYLAMINE	2	68	24	52	34	12
PENTACHLOROPHENOL	2	100	18	(C)	(C)	91
PHENANTHRENE	2	78	12	60	26	12
PHENOL	2	43	14	39	21	3
PYRENE	2	81	14	53	20	21
TRICHLOROBIPHENYL	2	74	12	123	14	-55
D10-ACENAPHTHENE (B)	2	73	17	60	23	13
D10-FLUORENE (B)	2	78	11	55	25	23
D10-PYRENE (B)	2	90	13	75	23	15
D3-2,4-DICHLOROPHENOL (B)	2	73	8	67	22	6

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD ADDED TO EACH FISH SAMPLE PRIOR TO EXTRACTION TO OBTAIN AN INDICATION OF METHOD PERFORMANCE.
(C) THE COMPOUND WAS NOT DETECTED.
(D) THE PEAK SATURATED THE DETECTOR.
(E) POSITIVE NUMBER INDICATES GREATER RECOVERY FROM SAMPLES ANALYZED IMMEDIATELY AFTER SPIKING.

unlikely that these priority pollutant compounds are actually present in the fish tissue analyzed. Instead these data indicate that some cross-contamination may have occurred in the GPC cleanup system during sample processing. An unspiked fish sample was processed with the spiked fish samples in the 24-hour storage study; low levels of only three compounds, anthracene, bis-(2-ethylhexyl) phthalate, and butylbenzyl phthalate, were found in the process blank. Recovery data corrected for levels of these compounds found in the process blank are given in Table 18. The source of contamination was successfully eliminated.

METHOD RANGE AND DETECTION LIMIT STUDIES

Prior to initiation of the method range and detection limit studies, collection of the GPC fraction was initiated at that time as signaled by the valley observed between elution of the corn oil and dioctylphenylphosphonate peaks of the GPC calibration mixture shown in Figure 6. Past experience with the catfish samples processed during the preliminary method evaluation studies indicated that these specifications resulted in quantitative recoveries of the phthalate compounds while eliminating most of the fish lipid material. During processing of the scallop-catfish-salmon mixed fish matrix used for method range studies it became apparent that high levels of lipid material were being carried over into the GPC fraction; this lipid material was identified as long-chain fatty acid material and made analysis of the samples by GC-MS impossible. The specifications for initiation of GPC fraction collection were changed such that fraction collection was started after elution of the dioctylphenylphosphonate peak when the intensity of the dioctylphenyl-phosphonate peak returned to 20 percent of baseline as indicated in Figure 6. Delaying fraction collection initiation resulted in drastically lowered fatty acid content while still allowing for quantitative recoveries of most of the priority pollutant compounds with the exception of the long-chain phthalates.

As specified in the Single Laboratory Validation Protocol, spiking levels used for method range studies were chosen to span the instrument range as determined during the instrument range studies. Recoveries obtained from replicate analyses of the mixed fish matrix spiked

at each of the spiking levels are given in Tables 20-24. A summary of the average recovery data obtained at each spiking level is given in Table 25. In general, recoveries obtained from the three highest spiking levels were greater than 50 percent and repeatable over the spiking range. Two exceptions were the extremely polar 2,4-dinitrophenol and 4-nitrophenol, which yielded recoveries lower than 35 percent, or were not recovered at all. Recoveries of the long-chain phthalates were low, presumably due to the fact that collection of the GPC fraction was postponed in order to eliminate late-eluting fatty acid material. Hexachlorocyclopentadiene recoveries were low; as previously mentioned, hexachlorocyclopentadiene is subject to decomposition in a hot injection port. Virtually none of the priority pollutant compounds were detected at the low spiking level and only a few were detected at the medium-low spiking level. Although many of the phthalates were detected at the two lowest spiking levels, recoveries were generally greater than 120 percent indicating that the compounds were likely contaminants introduced during sample processing.

MATRIX VALIDATION STUDIES

Whole minnows were used for the matrix validation studies in order to examine an entirely different fish matrix than previously used. The minnow sample extracts contained approximately the same extractable lipid content, 50-60 mg/g, as the matrices previously examined. However, the GPC chromatogram generated from the minnow extract indicated that high levels of material were still eluting after initiation of sample collection; this observation was confirmed by residue weight measurements. Apparently lipid composition varies depending on the specific fish being analyzed. For this reason, initiation of fraction collection was delayed for four minutes during the matrix validation studies using minnow samples. Delaying initiation of the fraction was found to result in low recoveries of the long-chain phthalates.

The Single Laboratory Validation Protocol specifies that the spiking levels used for the matrix validation studies be at the extremes of the method range as determined during the method range studies. The time constraints of the work assignment did not allow for generation of the method range data prior to initiation of the matrix validation studies.

TABLE 20. RESULTS FROM METHOD RANGE STUDIES CONDUCTED AT THE LOW CONCENTRATION LEVEL

COMPOUND	SPIKE LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE								AMOUNT FOUND IN BLANK, UG/G	AVG(A)	%RSD
		1	2	3	4	5	6	7	8			
1,2,4-TRICHLOROBENZENE	0.02	57	64	56	34	(C)	138	132	(C)	(C)	69	68
1,2-DICHLOROBENZENE	0.02	53	63	39	73	48	93	69	128	(C)	71	38
1,3-DICHLOROBENZENE	0.02	72	114	62	122	64	90	85	65	(C)	84	26
1,4-DICHLOROBENZENE	0.02	79	114	77	156	73	141	175	175	(C)	124	33
2,4,6-TRICHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DICHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DIMETHYLPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DINITROPHENOL	0.12	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DINITROTOLUENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,6-DINITROTOLUENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-CHLORONAPHTHALENE	0.02	131	66	78	72	54	101	114	116	(C)	92	28
2-CHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-METHYL-4,6-DINITROPHENOL	0.12	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-NITROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4,4'-DDD	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4,4'-DDE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4,4'-DDT	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-BROMOPHENYL PHENYL ETHER	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-CHLOROPHENYL PHENYL ETHER	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-CHLORO-3-METHYLPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-NITROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ACENAPHTHENE	0.02	105	106	94	134	118	129	184	145	0.010	78	21
ACENAPHTHYLENE	0.02	87	63	99	105	63	103	127	123	(C)	96	23
ALDRIN	0.02	(C)	177	196	108	(C)	179	321	(C)	(C)	140	49
ALPHA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ALPHA-CHLORDANE	0.12	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ANTHRACENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(A)ANTHRACENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(A)PYRENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(B)FLUORANTHENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(G,H,I,)PERYLENE	0.02	(C)	(C)	(_)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(K)FLUORANTHENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BETA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BIS(2-CHLOROETHYL) ETHER	0.02	61	81	93	210	(A)	115	119	154	(C)	105	50
BIS-2-CHLOROETHOXYMETHANE	0.02	106	37	112	132	202	(A)	(A)	26	(C)	102	58
BIS-(2-CHLOROISOPROPYL) ETHER	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BIS-(2-ETHYLHEXYL) PHTHALATE	0.02	22	150	1417	331	508	235	290	264	0.033	238	101
BUTYLBENZYL PHTHALATE	0.02	1125	1341	1287	1153	1685	924	5806	2267	0.254	655	75
CHRYSENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DELTA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIBENZO(A,H)ANTHRACENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIELDRIN	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIETHYL PHTHALATE	0.02	246	(A)	395	813	776	511	692	817	0.069	263	63
DIMETHYL PHTHALATE	0.02	(C)	104	201	217	136	243	284	327	(C)	216	34
DI-N-BUTYL PHTHALATE	0.02	162	289	230	257	356	367	481	(C)	(C)	(C)	32
DI-N-OCTYL PHTHALATE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDOSULFAN SULFATE	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDOSULFAN I	0.20	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDOSULFAN II	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDRIN	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDRIN ALDEHYDE	0.40	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
FLUORANTHENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
FLUORENE	0.02	114	104	107	128	104	131	132	137	0.015	44	11
GAMMA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
GAMMA-CHLORDANE	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEPTACHLOR	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEPTACHLOR EPOXIDE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROBENZENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROBUTADIENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROCYCLOPENTADIENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROETHANE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
INDENO(1,2,3-CD)PYRENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ISOPHORONE	0.02	49	79	95	126	88	98	11	(A)	(C)	78	45
NAPHTHALENE	0.02	240	157	131	133	142	188	180	154	0.023	52	21
NITROBENZENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
N-NITROSODIPHENYLAMINE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
N-NITROSODIPROPYLAMINE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PENTACHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PHENANTHRENE	0.02	180	176	160	192	147	225	238	249	0.029	51	18
PHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PYRENE	0.02	407	163	183	206	125	214	223	(C)	(C)	217	30
TRICHLOROBIPHENYL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
D10-ACENAPHTHENE (B)	2.00	(D)	(D)	91	111	(C)	41	112	133	-	98	32
D10-FLUORENE (B)	2.00	(D)	(D)	103	121	75	72	128	151	-	108	26
D10-PYRENE (B)	2.00	(D)	(D)	117	116	111	97	148	221	-	135	30
D3-2,4-DICHLOROPHENOL (B)	2.00	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	-	(C)	(C)

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD ADDED TO EACH FISH SAMPLE PRIOR TO EXTRACTION TO OBTAIN AN INDICATION OF METHOD PERFORMANCE.
(C) THE COMPOUND WAS NOT DETECTED.
(D) RECOVERY STANDARDS OMITTED FROM SAMPLE.

TABLE 21. RESULTS FROM METHOD RANGE STUDIES CONDUCTED AT THE MEDIUM-LOW CONCENTRATION LEVEL

COMPOUND	SPIKE LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE 1	2	3	4	5	6	7	8	AVG	%RSD	AVG(A)
1,2,4-TRICHLOROBENZENE	0.02	81	89	60	36	(C)	148	142	(C)	73	68	0
1,2-DICHLOROBENZENE	0.02	87	87	42	78	51	99	74	137	78	38	78
1,3-DICHLOROBENZENE	0.02	77	122	67	130	89	97	91	70	90	26	0
1,4-DICHLOROBENZENE	0.02	84	122	83	166	78	151	187	187	132	33	132
2,4,6-TRICHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DICHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DIMETHYLPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DINITROPHENOL	0.12	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DINITROTOLUENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,6-DINITROTOLUENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-CHLORONAPHTHALENE	0.02	140	70	85	77	56	108	122	124	98	28	98
2-CHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-METHYL-4,6-DINITROPHENOL	0.12	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-NITROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4,4'-DDD	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4,4'-DDE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4,4'-DDT	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-BROMOPHENYL PHENYL ETHER	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-CHLOROPHENYL PHENYL ETHER	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-CHLORO-3-METHYLPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-NITROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ACENAPHTHENE	0.02	113	113	100	143	128	138	187	155	136	21	82
ACENAPHTHYLENE	0.02	93	88	106	113	87	110	135	132	103	23	103
ALDRIN	0.02	(C)	189	210	116	(C)	182	343	(C)	150	49	150
ALPHA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ALPHA-CHLORDANE	0.12	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ANTHRACENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(A)ANTHRACENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(A)PYRENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(B)FLUORANTHENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(G,H,I,)PERYLENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(K)FLUORANTHENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BETA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BIS(2-CHLOROETHYL) ETHER	0.02	86	87	100	225	(A)	124	128	185	113	50	113
BIS-2-CHLOROETHOXYMETHANE	0.02	113	40	120	141	216	(A)	(A)	28	110	58	110
BIS-(2-CHLOROISOPROPYL) ETHER	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BIS-(2-ETHYLHEXYL) PHTHALATE	0.02	23	161	1517	354	544	251	311	283	430	101	255
BUTYLBENZYL PHTHALATE	0.02	1204	1435	1378	1234	1803	988	5999	2425	2058	75	700
CHRYSENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DELTA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIBENZO(A,H)ANTHRACENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIELDRIN	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIETHYL PHTHALATE	0.02	263	(A)	423	870	830	547	740	875	650	63	281
DIMETHYL PHTHALATE	0.02	(C)	111	215	232	146	260	303	350	231	34	231
DI-N-BUTYL PHTHALATE	0.02	173	288	246	275	381	393	515	(C)	324	32	0
DI-N-OCTYL PHTHALATE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDOSULFAN SULFATE	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDOSULFAN I	0.20	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDOSULFAN II	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDRIN	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDRIN ALDEHYDE	0.40	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
FLUORANTHENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
FLUORENE	0.02	122	112	116	137	111	140	141	147	128	11	47
GAMMA-BHC	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
GAMMA-CHLORDANE	0.10	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEPTACHLOR	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEPTACHLOR EPOXIDE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROBENZENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROBUTADIENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROCYCLOPENTADIENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROETHANE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
INDENO(1,2,3-CD)PYRENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ISOPHORONE	0.02	53	84	102	135	94	105	11	(A)	83	45	83
NAPHTHALENE	0.02	257	168	140	142	152	201	193	165	177	21	55
NITROBENZENE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
N-NITROSODIPHENYLAMINE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
N-NITROSODIPROPYLAMINE	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PENTACHLOROPHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PHENANTHRENE	0.02	192	188	171	205	157	241	255	267	210	18	54
PHENOL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PYRENE	0.02	436	174	196	220	134	229	239	(C)	232	38	232
TRICHLOROBIPHENYL	0.02	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
D10-ACENAPHTHENE (B)	2.00	(D)	(D)	98	118	(C)	44	119	142	104	32	
D10-FLUORENE (B)	2.00	(D)	(D)	110	129	80	77	137	181	116	26	
D10-PYRENE (B)	2.00	(D)	(D)	125	124	119	104	158	236	145	30	
D3-2,4-DICHLOROPHENOL (B)	2.00	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD.
(C) THE COMPOUND WAS NOT DETECTED.
(D) RECOVERY STANDARDS OMITTED FROM SAMPLE.

TABLE 22. RESULTS FROM METHOD RANGE STUDIES CONDUCTED AT THE MEDIUM CONCENTRATION LEVEL

	SPIKE LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE										
		1	2	3	4	5	6	7	8	AVG	%RSD	AVG(A)
1,2,4-TRICHLOROBENZENE	0.06	90	99	82	90	74	79	81	88	81	11	81
1,2-DICHLOROBENZENE	0.06	78	92	94	74	82	80	86	72	80	11	80
1,3-DICHLOROBENZENE	0.06	96	71	89	96	78	85	94	77	86	10	86
1,4-DICHLOROBENZENE	0.06	85	119	112	105	113	87	121	71	103	18	103
2,4,6-TRICHLOROPHENOL	0.06	74	50	60	(A)	38	42	38	(A)	50	28	50
2,4-DICHLOROPHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DIMETHYLPHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DINITROPHENOL	0.36	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DINITROTOLUENE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,6-DINITROTOLUENE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-CHLORONAPHTHALENE	0.06	86	101	91	81	82	83	91	79	87	8	87
2-CHLOROPHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-METHYL-4,6-DINITROPHENOL	0.36	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2-NITROPHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4,4'-DDD	0.06	144	158	118	98	95	161	117	127	127	18	127
4,4'-DDE	0.06	140	139	118	96	105	117	119	124	120	12	120
4,4'-DDT	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-BROMOPHENYL PHENYL ETHER	0.06	117	136	117	78	71	100	78	172	108	30	108
4-CHLOROPHENYL PHENYL ETHER	0.06	93	96	95	78	77	74	79	88	85	10	85
4-CHLORO-3-METHYLPHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
4-NITROPHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ACENAPHTHENE	0.06	93	103	91	80	89	91	94	93	92	6	91
ACENAPHTHYLENE	0.06	91	109	97	86	86	91	97	98	94	8	94
ALDRIN	0.06	130	112	110	105	119	131	112	114	116	8	116
ALPHA-BHC	0.06	97	101	85	85	83	80	89	80	83	15	83
ALPHA-CHLORDANE	0.36	81	91	83	63	58	67	74	100	77	17	77
ANTHRACENE	0.06	101	114	109	83	91	102	104	91	99	10	99
BENZO(A)ANTHRACENE	0.06	90	94	63	80	99	97	80	127	91	19	91
BENZO(A)PYRENE	0.06	134	86	46	(C)	128	126	112	(A)	109	30	109
BENZO(B)FLUORANTHENE	0.06	122	84	48	58	95	102	78	144	91	32	91
BENZO(G,H,I,)PERYLENE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BENZO(K)FLUORANTHENE	0.06	89	76	46	56	98	93	80	120	82	27	82
BETA-BHC	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
BIS(2-CHLOROETHYL) ETHER	0.06	82	74	68	65	84	78	81	63	74	10	74
BIS-2-CHLOROETHOXYMETHANE	0.06	119	131	108	110	109	101	112	75	108	14	108
BIS-(2-CHLOROISOPROPYL) ETHER	0.06	98	82	98	39	91	107	85	100	85	25	85
BIS-(2-ETHYLHEXYL) PHTHALATE	0.06	142	110	72	65	15	115	279	114	114	63	112
BUTYLBENZYL PHTHALATE	0.06	445	634	408	425	811	291	346	866	503	35	490
CHRYSENE	0.06	133	70	94	46	146	143	118	109	107	31	107
DELTA-BHC	0.06	131	286	161	134	103	158	101	178	157	35	152
DIBENZO(A,H)ANTHRACENE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIELDRIN	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
DIETHYL PHTHALATE	0.06	186	184	181	114	166	208	183	136	170	17	168
DIMETHYL PHTHALATE	0.06	89	97	88	76	80	86	81	90	86	7	86
DI-N-BUTYL PHTHALATE	0.06	178	169	165	148	178	173	162	155	166	6	156
DI-N-OCTYL PHTHALATE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ENDOSULFAN SULFATE	0.30	124	148	84	72	83	117	92	121	105	23	105
ENDOSULFAN I	0.60	163	216	157	140	115	272	195	148	176	27	176
ENDOSULFAN II	0.30	110	128	88	85	111	149	120	153	118	20	118
ENDRIN	0.30	88	91	79	73	97	127	114	85	92	20	92
ENDRIN ALDEHYDE	1.20	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
FLUORANTHENE	0.06	111	113	102	84	113	124	110	114	109	10	109
FLUORENE	0.06	102	107	106	87	97	97	100	106	100	6	99
GAMMA-BHC	0.06	75	89	75	61	81	89	59	58	75	19	75
GAMMA-CHLORDANE	0.30	114	124	99	89	73	87	93	100	97	15	97
HEPTACHLOR	0.06	74	136	131	110	69	94	114	99	103	22	103
HEPTACHLOR EPOXIDE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROBENZENE	0.06	111	150	77	77	84	80	77	105	93	28	93
HEXACHLOROBUTADIENE	0.06	92	93	80	77	81	58	67	77	78	16	78
HEXACHLOROCYCLOPENTADIENE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROETHANE	0.06	75	77	88	77	67	66	65	69	73	10	73
INDENO(1,2,3-CD)PYRENE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ISOPHORONE	0.06	78	89	67	90	81	72	86	82	78	13	78
NAPHTHALENE	0.06	106	112	105	101	109	106	110	89	105	6	103
NITROBENZENE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
N-NITROSODIPHENYLAMINE	0.06	80	103	97	65	70	65	81	110	84	19	79
N-NITROSODIPROPYLAMINE	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PENTACHLOROPHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PHENANTHRENE	0.06	122	122	115	104	115	120	108	118	115	5	114
PHENOL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
PYRENE	0.06	114	139	103	101	126	132	137	145	125	13	125
TRICHLOROBIPHENYL	0.06	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
D10-ACENAPHTHENE (B)	2.00	89	96	83	71	75	74	88	96	85	12	
D10-FLUORENE (B)	2.00	107	99	92	73	82	86	95	108	93	12	
D10-PYRENE (B)	2.00	109	126	91	73	104	106	99	129	105	16	
D3-2,4-DICHLOROPHENOL (B)	2.00	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD.
(C) THE COMPOUND WAS NOT DETECTED.

TABLE 23. RESULTS FROM METHOD RANGE STUDIES CONDUCTED AT THE MEDIUM-HIGH CONCENTRATION LEVEL

COMPOUND	SPIKED LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE								AVG	%RSD	AVG(A)
		1	2	3	4	5	6	7	8			
1,2,4-TRICHLOROBENZENE	0.2	57	43	53	49	71	66	56	88	61	22	55
1,2-DICHLOROBENZENE	0.2	61	38	60	61	72	58	51	88	61	22	61
1,3-DICHLOROBENZENE	0.2	64	56	5	76	83	76	56	106	65	42	55
1,4-DICHLOROBENZENE	0.2	46	47	42	71	81	65	59	82	59	22	59
2,4,6-TRICHLOROPHENOL	0.2	71	52	87	84	73	81	76	92	76	17	76
2,4-DICHLOROPHENOL	0.2	81	42	63	80	71	81	71	110	76	24	76
2,4-DIMETHYLPHENOL	0.2	135	126	200	93	226	246	190	126	188	31	188
2,4-DINITROPHENOL	1.2	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
2,4-DINITROTOLUENE	0.2	67	54	81	43	58	99	58	96	67	28	67
2,6-DINITROTOLUENE	0.2	65	38	59	54	73	90	71	98	68	26	68
2-CHLORONAPHTHALENE	0.2	61	41	60	81	65	71	84	98	67	23	67
2-CHLOROPHENOL	0.2	72	33	53	58	58	84	42	109	61	35	61
2-METHYL-4,6-DINITROPHENOL	1.2	(C)	(C)	(C)	30	45	84	45	56	52	35	52
2-NITROPHENOL	0.2	42	56	81	68	81	109	60	87	72	29	72
4,4'-DDD	0.2	97	84	83	89	113	103	88	136	94	23	94
4,4'-DDE	0.2	133	111	126	184	97	83	78	115	113	23	113
4,4'-DDT	0.2	(C)	(C)	(C)	(C)	80	144	56	321	150	69	150
4-BROMOPHENYL PHENYL ETHER	0.2	85	80	85	84	83	81	63	89	81	19	81
4-CHLOROPHENYL PHENYL ETHER	0.2	(A)	46	51	85	80	80	62	89	68	45	68
4-CHLORO-3-METHYLPHENOL	0.2	131	87	103	103	103	120	108	133	112	12	112
4-NITROPHENOL	0.2	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ACENAPHTHENE	0.2	98	98	113	101	64	72	65	100	89	20	84
ACENAPHTHYLENE	0.2	80	48	87	75	69	78	70	100	73	20	73
ALDRIN	0.2	97	68	116	126	74	55	57	105	87	30	87
ALPHA-BHC	0.2	51	81	64	83	85	83	69	102	72	21	72
ALPHA-CHLORDANE	1.2	102	73	100	109	84	70	51	104	87	22	87
ANTHRACENE	0.2	85	91	89	96	88	82	70	101	80	17	80
BENZO(A)ANTHRACENE	0.2	79	54	98	149	116	103	88	141	103	29	97
BENZO(A)PYRENE	0.2	140	78	127	148	153	124	95	163	129	21	121
BENZO(B)FLUORANTHENE	0.2	72	71	113	82	142	119	93	170	108	31	108
BENZO(G,H,I,)PERYLENE	0.2	(C)	(C)	(C)	(C)	54	38	40	55	47	17	47
BENZO(K)FLUORANTHENE	0.2	70	57	84	90	127	109	89	152	97	30	97
BETA-BHC	0.2	36	31	37	54	70	73	67	91	57	35	57
BIS(2-CHLOROETHYL) ETHER	0.2	63	55	54	84	63	81	56	73	63	15	63
BIS-2-CHLOROETHOXYMETHANE	0.2	79	58	86	92	63	57	56	91	73	20	73
BIS-(2-CHLOROISOPROPYL) ETHER	0.2	83	64	75	128	84	40	32	83	71	39	71
BIS-(2-ETHYLHEXYL) PHTHALATE	0.2	79	80	72	144	60	50	36	113	79	41	21
BUTYLBENZYL PHTHALATE	0.2	117	129	183	265	127	137	141	403	188	50	66
CHRYSENE	0.2	183	156	225	107	107	98	81	132	136	33	130
DELTA-BHC	0.2	61	(C)	100	208	56	75	63	94	94	52	69
DIBENZO(A,H)ANTHRACENE	0.2	136	(C)	120	133	201	166	126	316	171	38	171
DIELDRIN	0.2	73	72	119	87	57	50	46	81	73	30	73
DIETHYL PHTHALATE	0.2	158	121	186	193	54	76	69	135	124	40	104
DIMETHYL PHTHALATE	0.2	52	34	57	57	47	67	57	86	57	25	57
DI-N-BUTYL PHTHALATE	0.2	108	83	105	162	85	96	90	51	98	30	16
DI-N-OCTYL PHTHALATE	0.2	23	31	41	43	30	25	27	48	33	26	27
ENDOSULFAN SULFATE	1.0	72	60	76	92	103	86	72	127	86	23	86
ENDOSULFAN I	2.0	189	67	203	343	100	98	90	178	159	53	159
ENDOSULFAN II	1.0	46	34	71	86	93	83	70	115	75	32	75
ENDRIN	1.0	320	190	398	505	89	90	86	146	228	66	228
ENDRIN ALDEHYDE	4.0	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
FLUORANTHENE	0.2	62	51	64	55	81	94	74	114	75	26	75
FLUORENE	0.2	57	57	67	62	65	77	72	98	69	18	59
GAMMA-BHC	0.2	52	88	83	74	75	87	70	106	77	19	77
GAMMA-CHLORDANE	1.0	100	84	107	239	113	83	68	146	118	43	118
HEPTACHLOR	0.2	99	82	95	89	70	78	70	94	85	12	85
HEPTACHLOR EPOXIDE	0.2	53	30	119	173	110	50	129	83	93	48	93
HEXACHLOROBENZENE	0.2	102	72	73	100	61	73	61	86	78	19	78
HEXACHLOROBUTADIENE	0.2	67	49	72	63	71	63	57	88	66	16	66
HEXACHLOROCYCLOPENTADIENE	0.2	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
HEXACHLOROETHANE	0.2	61	49	50	76	70	80	54	94	84	22	64
INDENO(1,2,3-CD)PYRENE	0.2	63	(A)	136	89	196	146	120	283	129	62	129
ISOPHORONE	0.2	58	48	62	83	49	54	50	78	60	21	60
NAPHTHALENE	0.2	64	49	69	74	75	63	59	120	72	28	62
NITROBENZENE	0.2	79	53	72	91	87	81	63	110	80	21	80
N-NITROSODIPHENYLAMINE	0.2	79	45	60	87	53	85	72	81	68	21	34
N-NITROSODIPROPYLAMINE	0.2	80	88	102	170	85	62	52	98	92	36	92
PENTACHLOROPHENOL	0.2	(C)	(C)	(C)	(C)	123	133	119	117	123	5	123
PHENANTHRENE	0.2	66	62	66	90	68	78	68	105	75	18	59
PHENOL	0.2	88	82	67	108	84	89	57	(A)	77	23	77
PYRENE	0.2	108	83	150	32	105	99	92	132	100	33	100
TRICHLOROBIPHENYL	0.2	(C)	(C)	(C)	(C)	73	76	60	94	76	16	76
D10-ACENAPHTHENE (B)	2.0	62	45	51	69	57	71	85	253	84	77	
D10-FLUORENE (B)	2.0	56	56	85	68	58	74	68	263	89	74	
D10-PYRENE (B)	2.0	94	109	116	10	82	88	82	334	114	77	
D3-2,4-DICHLOROPHENOL (B)	2.0	50	57	78	82	68	75	67	256	89	72	

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD.
(C) THE COMPOUND WAS NOT DETECTED.

TABLE 24. RESULTS FROM METHOD RANGE STUDIES CONDUCTED AT THE HIGH CONCENTRATION LEVEL

COMPOUND	SPIKE LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE 1	2	3	4	5	6	7	AVG	%RSD	AVG(A)
1,2,4-TRICHLOROBENZENE	0.6	110	95	103	72	64	82	86	87	17	85
1,2-DICHLOROBENZENE	0.6	117	98	98	72	63	84	82	84	22	84
1,3-DICHLOROBENZENE	0.6	138	111	119	72	67	82	47	91	33	86
1,4-DICHLOROBENZENE	0.6	122	128	137	71	63	72	53	92	35	92
2,4,6-TRICHLOROPHENOL	0.6	111	108	116	66	64	84	82	90	22	90
2,4-DICHLOROPHENOL	0.6	118	110	118	71	60	76	85	91	24	91
2,4-DIMETHYLPHENOL	0.6	96	89	94	101	137	92	114	112	23	112
2,4-DINITROPHENOL	3.6	47	51	49	12	16	(C)	(C)	35	49	35
2,4-DINITROTOLUENE	0.6	112	110	115	66	63	79	78	89	23	89
2,6-DINITROTOLUENE	0.6	109	112	108	71	58	82	74	88	23	88
2-CHLORONAPHTHALENE	0.6	114	107	112	71	66	83	88	91	20	91
2-CHLOROPHENOL	0.6	117	88	93	66	59	74	71	81	23	81
2-METHYL-4,6-DINITROPHENOL	3.6	74	80	76	35	42	53	48	58	29	58
2-NITROPHENOL	0.6	100	89	105	67	63	65	73	80	20	80
4,4'-DDD	0.6	123	116	116	91	90	135	114	112	14	112
4,4'-DDE	0.6	112	102	105	92	83	128	99	103	13	103
4,4'-DDT	0.6	159	156	158	84	81	164	139	134	25	134
4-BROMOPHENYL PHENYL ETHER	0.6	118	104	102	71	70	98	81	92	18	92
4-CHLOROPHENYL PHENYL ETHER	0.6	123	107	110	68	64	91	82	92	23	92
4-CHLORO-3-METHYLPHENOL	0.6	124	122	118	74	79	109	123	107	18	107
4-NITROPHENOL	0.6	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ACENAPHTHENE	0.6	110	103	108	71	64	84	89	90	18	88
ACENAPHTHYLENE	0.6	112	109	113	73	67	87	90	93	19	93
ALDRIN	0.6	115	96	106	80	62	87	109	93	19	93
ALPHA-BHC	0.6	118	108	107	73	71	86	81	92	18	92
ALPHA-CHLORDANE	3.6	109	98	100	71	69	124	82	90	24	90
ANTHRACENE	0.6	121	110	106	73	70	96	88	95	18	95
BENZO(A)ANTHRACENE	0.6	97	109	116	96	95	131	117	109	12	107
BENZO(A)PYRENE	0.6	121	126	140	97	98	110	112	115	13	112
BENZO(B)FLUORANTHENE	0.6	96	98	112	101	101	157	114	111	18	111
BENZO(G,H,I,)PERYLENE	0.6	32	43	58	33	39	48	35	41	21	41
BENZO(K)FLUORANTHENE	0.6	113	121	124	90	96	127	108	111	12	111
BETA-BHC	0.6	128	126	128	82	67	88	79	100	25	100
BIS(2-CHLOROETHYL) ETHER	0.6	101	85	93	59	64	68	59	78	21	76
BIS-2-CHLOROETHOXYMETHANE	0.6	104	91	99	68	57	71	87	82	20	82
BIS-(2-CHLOROISOPROPYL) ETHER	0.6	71	59	63	75	65	73	87	71	12	71
BIS-(2-ETHYLHEXYL) PHTHALATE	0.6	55	62	44	25	15	24	31	37	44	17
BUTYLBENZYL PHTHALATE	0.6	168	165	164	127	118	187	197	161	17	122
CHRYSENE	0.6	148	141	157	95	92	126	107	124	20	122
DELTA-BHC	0.6	122	114	105	75	65	92	94	95	20	88
DIBENZO(A,H)ANTHRACENE	0.6	108	114	119	124	141	185	156	135	19	135
DIELDRIN	0.6	90	87	91	73	64	83	102	84	14	84
DIETHYL PHTHALATE	0.6	122	114	109	71	65	84	98	95	21	89
DIMETHYL PHTHALATE	0.6	98	95	90	55	53	72	77	77	22	77
DI-N-BUTYL PHTHALATE	0.6	128	106	105	80	72	83	104	97	18	53
DI-N-OCTYL PHTHALATE	0.6	19	17	23	15	15	19	21	19	15	17
ENDOSULFAN SULFATE	3	110	101	105	77	79	111	88	96	14	96
ENDOSULFAN I	6	128	121	120	86	74	119	113	109	17	109
ENDOSULFAN II	3	109	105	102	77	69	100	97	94	15	94
ENDRIN	3	120	120	120	92	83	97	124	108	14	108
ENDRIN ALDEHYDE	12	(A)	(A)	(A)	30	22	34	44	33	25	33
FLUORANTHENE	0.6	124	117	113	79	76	112	100	103	17	103
FLUORENE	0.6	118	107	108	71	65	87	87	91	20	88
GAMMA-BHC	0.6	96	87	107	81	62	78	97	87	18	87
GAMMA-CHLORDANE	3	128	116	130	83	81	138	82	110	20	110
HEPTACHLOR	0.6	117	110	109	69	63	85	100	93	21	93
HEPTACHLOR EPOXIDE	0.6	96	93	95	95	89	108	98	96	6	96
HEXACHLOROBENZENE	0.6	109	96	94	63	60	102	69	85	22	85
HEXACHLOROBUTADIENE	0.6	102	98	105	68	64	86	76	86	18	86
HEXACHLOROCYCLOPENTADIENE	0.6	(C)	12	9	(C)	(C)	18	15	13	23	13
HEXACHLOROETHANE	0.6	107	90	98	75	66	75	72	83	17	83
INDENO(1,2,3-CD)PYRENE	0.6	152	158	183	130	132	163	144	152	11	152
ISOPHORONE	0.6	94	84	93	59	53	67	75	75	20	75
NAPHTHALENE	0.6	111	100	108	75	66	91	97	93	17	88
NITROBENZENE	0.6	105	85	82	63	55	83	81	79	19	79
N-NITROSODIPHENYLAMINE	0.6	115	117	113	75	73	78	77	92	21	79
N-NITROSODIPROPYLAMINE	0.6	76	84	96	67	60	54	78	73	18	73
PENTACHLOROPHENOL	0.6	120	100	100	69	66	139	112	101	24	101
PHENANTHRENE	0.6	128	115	115	74	69	93	92	98	21	92
PHENOL	0.6	98	103	82	61	44	63	(A)	75	28	75
PYRENE	0.6	137	127	126	89	84	123	102	112	17	112
TRICHLOROBIPHENYL	0.6	112	103	112	69	67	99	77	91	20	91
D10-ACENAPHTHENE (B)	2	118	118	112	136	61	(D)	24	82	59	
D10-FLUORENE (B)	2	121	116	114	136	60	(D)	24	82	60	
D10-PYRENE (B)	2	131	128	121	158	72	(D)	26	91	59	
D3-2,4-DICHLOROPHENOL (B)	2	122	123	123	130	82	(D)	29	98	55	

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD.
(C) THE COMPOUND WAS NOT DETECTED.
(D) RECOVERY STANDARDS OMITTED FROM SAMPLE.

TABLE 25. SUMMARY OF RESULTS FROM METHOD RANGE STUDIES

COMPOUND	AVERAGE PERCENT RECOVERY AT GIVEN SPIKE LEVEL (A)				
	HIGH	MED-HIGH	MEDIUM	MED-LOW	LOW
1,2,4-TRICHLOROBENZENE	63	79	51	75	(C)
1,2-DICHLOROBENZENE	66	79	57	74	71
1,3-DICHLOROBENZENE	66	80	52	79	(C)
1,4-DICHLOROBENZENE	67	86	55	96	124
2,4,6-TRICHLOROPHENOL	62	84	71	47	(C)
2,4-DICHLOROPHENOL	62	85	71	(C)	(C)
2,4-DIMETHYLPHENOL	171	104	157	(C)	(C)
2,4-DINITROPHENOL	13	33	(C)	(C)	(C)
2,4-DINITROTOLUENE	54	83	63	(C)	(C)
2,6-DINITROTOLUENE	58	82	64	(C)	(C)
2-CHLORONAPHTHALENE	61	85	63	81	92
2-CHLOROPHENOL	59	76	57	(C)	(C)
2-METHYL-4,6-DINITROPHENOL	36	54	48	(C)	(C)
2-NITROPHENOL	56	75	67	(C)	(C)
4,4'-DDD	70	105	88	119	(C)
4,4'-DDE	65	96	108	112	(C)
4,4'-DDT	56	125	141	(C)	(C)
4-BROMOPHENYL PHENYL ETHER	60	86	76	101	(C)
4-CHLOROPHENYL PHENYL ETHER	60	86	63	80	(C)
4-CHLORO-3-METHYLPHENOL	65	100	105	(C)	(C)
4-NITROPHENOL	15	(C)	(C)	(C)	(C)
ACENAPHTHENE	61	82	78	85	76
ACENAPHTHYLENE	64	87	69	88	96
ALDRIN	62	87	81	109	140
ALPHA-BHC	60	86	67	77	(C)
ALPHA-CHLORDANE	57	84	81	72	(C)
ANTHRACENE	62	89	75	93	(C)
BENZO(A)ANTHRACENE	69	100	90	85	(C)
BENZO(A)PYRENE	61	105	113	102	(C)
BENZO(B)FLUORANTHENE	63	104	101	85	(C)
BENZO(G,H,I,)PERYLENE	23	38	44	(C)	(C)
BENZO(K)FLUORANTHENE	64	104	91	77	(C)
BETA-BHC	61	93	54	(C)	(C)
BIS(2-CHLOROETHYL) ETHER	54	71	59	69	105
BIS-2-CHLOROETHOXYMETHANE	57	77	68	101	102
BIS-(2-CHLOROISOPROPYL) ETHER	53	66	66	79	(C)
BIS-(2-ETHYLHEXYL) PHTHALATE	11	16	20	105	238
BUTYLBENZYL PHTHALATE	79	114	62	458	655
CHRYSENE	66	114	122	100	(C)
DELTA-BHC	61	80	65	142	(C)
DIBENZO(A,H)ANTHRACENE	91	126	160	(C)	(C)
DIELDRIN	61	79	68	(C)	(C)
DIETHYL PHTHALATE	60	83	97	157	263
DIMETHYL PHTHALATE	50	72	53	80	216
DI-N-BUTYL PHTHALATE	50	49	15	145	(C)
DI-N-OCTYL PHTHALATE	8	16	26	(C)	(C)
ENDOSULFAN SULFATE	63	90	80	98	(C)
ENDOSULFAN I	65	102	148	165	(C)
ENDOSULFAN II	62	88	70	110	(C)
ENDRIN	73	101	213	86	(C)
ENDRIN ALDEHYDE	20	31	(C)	(C)	(C)
FLUORANTHENE	65	96	70	102	(C)
FLUORENE	61	82	55	92	44
GAMMA-BHC	64	81	72	70	(C)
GAMMA-CHLORDANE	64	103	110	91	(C)
HEPTACHLOR	58	87	79	97	(C)
HEPTACHLOR EPOXIDE	63	90	87	(C)	(C)
HEXACHLOROBENZENE	58	79	73	87	(C)
HEXACHLOROBUTADIENE	61	80	62	71	(C)
HEXACHLOROCYCLOPENTADIENE	13	13	(C)	(C)	(C)
HEXACHLOROETHANE	64	78	60	68	(C)
INDENO(1,2,3-CD)PYRENE	111	142	121	(C)	(C)
ISOPHORONE	52	70	56	73	78
NAPHTHALENE	65	82	58	97	52
NITROBENZENE	60	74	74	(C)	(C)
N-NITROSODIPHENYLAMINE	59	74	32	74	(C)
N-NITROSODIPROPYLAMINE	54	69	86	(C)	(C)
PENTACHLOROPHENOL	68	94	115	(C)	(C)
PHENANTHRENE	60	86	55	107	51
PHENOL	38	70	72	(C)	(C)
PYRENE	66	105	94	116	217
TRICHLOROBIPHENYL	60	85	71	(C)	(C)
D10-ACENAPHTHENE (B)	57	76	78	80	98
D10-FLUORENE (B)	57	77	83	87	108
D10-PYRENE (B)	62	85	107	98	135
D3-2,4-DICHLOROPHENOL (B)	57	92	83	(C)	(C)

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD ADDED TO EACH FISH SAMPLE PRIOR TO EXTRACTION TO OBTAIN AN INDICATION OF METHOD PERFORMANCE.

Since method range data were not available, spiking levels used for the matrix validation studies were those used for the low and high spiking levels of the method range studies. As previously described, method range data generated from fish spiked at the low concentration level were incomplete. Since it was unlikely that data obtained from the analysis of the low level matrix validation samples would provide useful data, these samples were not analyzed by GC-MS. Recoveries obtained from replicate analyses of the minnow matrix spiked at the high level are given in Table 26. In most cases, recoveries of priority compounds from the minnow samples did not vary significantly from those observed from the mixed fish matrix. A comparison of the recovery data obtained from the two fish matrixes is shown in Table 27. Recovery of di-n-butyl phthalate from the minnow samples was significantly decreased, presumably due to the fact that collection of the GPC fraction was initiated later than was done when the mixed fish matrix was processed.

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TABLE 26. RESULTS FROM MATRIX VALIDATION STUDIES CONDUCTED AT THE HIGH CENCENTRATION LEVEL

	SPIKED LEVEL, UG/G	PERCENT RECOVERY IN GIVEN SAMPLE										
		1	2	3	4	5	6	7	8	AVG	%RSD	AVG(A)
1,2,4-TRICHLOROBENZENE	2	77	78	75	70	78	85	81	78	75	6	75
1,2-DICHLOROBENZENE	2	79	77	75	71	73	86	76	81	75	6	75
1,3-DICHLOROBENZENE	2	104	103	101	102	120	95	134	127	111	12	111
1,4-DICHLOROBENZENE	2	106	104	88	96	118	98	121	116	106	10	106
2,4,6-TRICHLOROPHENOL	2	73	72	70	70	72	68	71	68	70	2	70
2,4-DICHLOROPHENOL	2	79	79	78	73	75	86	80	78	78	8	78
2,4-DIMETHYLPHENOL	2	72	70	73	57	42	42	57	47	58	21	58
2,4-DINITROPHENOL	12	24	29	30	33	27	31	33	22	29	14	29
2,4-DINITROTOLUENE	2	72	78	80	77	78	84	83	78	78	5	78
2,6-DINITROTOLUENE	2	77	77	77	76	78	79	86	84	79	5	79
2-CHLORONAPHTHALENE	2	83	81	81	77	79	74	88	84	81	5	81
2-CHLOROPHENOL	2	78	74	70	80	78	63	92	93	78	12	78
2-METHYL-4,6-DINITROPHENOL	12	50	59	64	64	51	62	62	56	58	9	58
2-NITROPHENOL	2	68	68	68	68	67	63	78	74	69	7	69
4,4'-DDD	2	95	92	93	86	101	98	104	97	96	6	96
4,4'-DDE	2	87	81	88	81	90	92	96	91	88	6	88
4,4'-DDT	2	88	83	89	83	85	79	95	85	86	5	85
4-BROMOPHENYL PHENYL ETHER	2	84	78	84	79	86	79	86	83	82	4	82
4-CHLOROPHENYL PHENYL ETHER	2	84	79	80	77	80	76	86	82	81	4	81
4-CHLORO-3-METHYLPHENOL	2	86	85	84	80	79	76	87	85	83	4	80
4-NITROPHENOL	2	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)	(C)
ACENAPHTHENE	2	83	84	83	76	77	71	92	86	81	7	81
ACENAPHTHYLENE	2	86	85	85	79	79	76	93	83	83	6	83
ALDRIN	2	83	91	86	79	83	93	123	109	93	15	93
ALPHA-BHC	2	83	79	82	79	79	84	86	86	82	3	82
ALPHA-CHLORDANE	12	89	76	87	84	85	92	89	84	86	5	86
ANTHRACENE	2	80	79	83	75	77	72	88	78	79	6	79
BENZO(A)ANTHRACENE	2	93	91	94	87	102	98	111	99	97	7	97
BENZO(A)PYRENE	2	90	83	94	80	85	90	106	79	88	9	87
BENZO(B)FLUORANTHENE	2	90	82	88	85	108	97	106	98	94	10	94
BENZO(G,H,I,)PERYLENE	2	45	42	45	37	46	55	55	40	46	13	46
BENZO(K)FLUORANTHENE	2	87	80	88	83	95	103	107	96	92	9	92
BETA-BHC	2	88	90	86	82	87	109	92	82	88	10	88
BIS(2-CHLOROETHYL) ETHER	2	66	90	68	75	67	69	92	88	79	13	79
BIS-2-CHLOROETHOXYMETHANE	2	71	78	72	65	68	69	86	82	74	9	74
BIS-(2-CHLOROISOPROPYL) ETHER	2	89	65	55	58	56	80	83	113	75	26	75
BIS-(2-ETHYLHEXYL) PHTHALATE	2	27	16	21	15	60	21	24	24	26	51	19
BUTYLBENZYL PHTHALATE	2	68	77	75	78	77	76	87	90	78	8	72
CHRYSENE	2	89	83	91	84	98	98	112	98	94	9	94
DELTA-BHC	2	85	82	87	77	86	94	95	94	87	7	87
DIBENZO(A,H)ANTHRACENE	2	108	120	118	111	139	146	150	107	125	13	125
DIELDRIN	2	80	86	86	75	81	87	113	107	89	14	89
DIETHYL PHTHALATE	2	67	65	64	61	61	60	75	72	66	8	62
DIMETHYL PHTHALATE	2	68	68	68	64	65	64	72	68	67	4	67
DI-N-BUTYL PHTHALATE	2	6	8	6	10	10	8	5	7	7	23	1
DI-N-OCTYL PHTHALATE	2	12	10	12	9	14	11	11	11	11	11	11
ENDOSULFAN SULFATE	10	89	78	85	81	86	87	91	86	86	5	86
ENDOSULFAN I	20	84	80	83	76	81	76	99	99	85	10	85
ENDOSULFAN II	10	83	82	86	79	87	81	95	91	86	6	86
ENDRIN	10	94	100	102	89	80	88	112	106	96	10	94
ENDRIN ALDEHYDE	40	47	56	51	43	45	46	71	65	53	18	53
FLUORANTHENE	2	89	87	89	84	98	94	100	92	91	5	91
FLUORENE	2	82	84	84	79	82	77	93	87	83	6	82
GAMMA-BHC	2	82	80	76	78	76	81	95	83	81	7	81
GAMMA-CHLORDANE	10	84	73	80	79	87	85	94	83	83	7	83
HEPTACHLOR	2	84	87	88	82	80	89	99	96	88	7	88
HEPTACHLOR EPOXIDE	2	76	86	84	77	77	90	109	101	87	13	87
HEXACHLOROBENZENE	2	84	73	79	82	79	80	80	75	79	4	79
HEXACHLOROBOTADIENE	2	80	75	75	73	73	72	76	76	75	3	75
HEXACHLOROCYCLOPENTADIENE	2	14	17	19	22	23	9	11	9	15	34	15
HEXACHLOROETHANE	2	76	77	76	68	72	77	89	85	78	8	78
INDENO(1,2,3-CD)PYRENE	2	96	95	108	94	90	130	87	107	101	13	101
ISOPHORONE	2	37	42	40	37	39	40	49	48	41	11	41
NAPHTHALENE	2	79	81	80	72	75	70	92	88	80	9	78
NITROBENZENE	2	76	73	68	70	81	80	90	88	78	9	78
N-NITROSODIPHENYLAMINE	2	72	71	70	62	58	58	73	67	67	9	67
N-NITROSODIPROPYLAMINE	2	56	67	62	55	55	62	79	74	64	13	61
PENTACHLOROPHENOL	2	74	69	74	65	77	70	67	57	69	8	69
PHENANTHRENE	2	86	83	84	79	85	71	94	87	84	7	81
PHENOL	2	42	58	54	43	46	40	77	63	53	23	53
PYRENE	2	88	83	88	81	92	83	96	90	87	6	87
TRICHLOROBIPHENYL	2	83	75	80	78	82	74	86	81	80	5	80
D10-ACENAPHTHENE (B)	2	73	72	75	71	77	64	83	76	74	7	
D10-FLUORENE (B)	2	73	73	75	72	81	71	85	78	76	6	
D10-PYRENE (B)	2	77	73	80	76	91	85	91	82	82	8	
D3-2,4-DICHLOROPHENOL (B)	2	66	68	68	67	72	59	74	69	68	6	

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD.
(C) THE COMPOUND WAS NOT DETECTED.

TABLE 27. COMPARISON OF RESULTS FROM HIGH LEVEL METHOD RANGE AND MATRIX VALIDATION STUDIES

COMPOUND	SPIKE LEVEL, UG/G	AVERAGE PERCENT RECOVERY IN GIVEN SAMPLE (A)	
		METHOD RANGE	METHOD VALIDATION
1,2,4-TRICHLOROBENZENE	2	63	70
1,2-DICHLOROBENZENE	2	66	70
1,3-DICHLOROBENZENE	2	66	103
1,4-DICHLOROBENZENE	2	67	99
2,4,6-TRICHLOROPHENOL	2	62	66
2,4-DICHLOROPHENOL	2	62	71
2,4-DIMETHYLPHENOL	2	171	54
2,4-DINITROPHENOL	12	13	27
2,4-DINITROTOLUENE	2	54	73
2,6-DINITROTOLUENE	2	58	74
2-CHLORONAPHTHALENE	2	61	76
2-CHLOROPHENOL	2	59	73
2-METHYL-4,6-DINITROPHENOL	12	36	55
2-NITROPHENOL	2	56	64
4,4'-DDD	2	70	89
4,4'-DDE	2	65	82
4,4'-DDT	2	56	79
4-BROMOPHENYL PHENYL ETHER	2	60	77
4-CHLOROPHENYL PHENYL ETHER	2	60	75
4-CHLORO-3-METHYLPHENOL	2	65	75
4-NITROPHENOL	2	15	(C)
ACENAPHTHENE	2	61	76
ACENAPHTHYLENE	2	64	78
ALDRIN	2	62	87
ALPHA-BHC	2	60	77
ALPHA-CHLORDANE	12	57	80
ANTHRACENE	2	62	74
BENZO(A)ANTHRACENE	2	69	91
BENZO(A)PYRENE	2	61	82
BENZO(B)FLUORANTHENE	2	63	88
BENZO(G,H,I,)PERYLENE	2	23	43
BENZO(K)FLUORANTHENE	2	64	86
BETA-BHC	2	61	82
BIS(2-CHLOROETHYL) ETHER	2	54	74
BIS-2-CHLOROETHOXYMETHANE	2	57	69
BIS-(2-CHLOROISOPROPYL) ETHER	2	53	70
BIS-(2-ETHYLHEXYL) PHTHALATE	2	11	18
BUTYLBENZYL PHTHALATE	2	79	67
CHRYSENE	2	66	88
DELTA-BHC	2	61	82
DIBENZO(A,H)ANTHRACENE	2	91	117
DIELDRIN	2	61	84
DIETHYL PHTHALATE	2	60	58
DIMETHYL PHTHALATE	2	50	63
DI-N-BUTYL PHTHALATE	2	50	1
DI-N-OCTYL PHTHALATE	2	8	11
ENDOSULFAN SULFATE	10	63	80
ENDOSULFAN I	20	65	79
ENDOSULFAN II	10	62	80
ENDRIN	10	73	88
ENDRIN ALDEHYDE	40	20	49
FLUORANTHENE	2	65	85
FLUORENE	2	61	77
GAMMA-BHC	2	64	76
GAMMA-CHLORDANE	10	64	77
HEPTACHLOR	2	58	82
HEPTACHLOR EPOXIDE	2	63	82
HEXACHLOROBENZENE	2	58	74
HEXACHLOROBUTADIENE	2	61	70
HEXACHLOROCYCLOPENTADIENE	2	13	14
HEXACHLOROETHANE	2	64	73
INDENO(1,2,3-CD)PYRENE	2	111	94
ISOPHORONE	2	52	39
NAPHTHALENE	2	65	73
NITROBENZENE	2	60	73
N-NITROSODIPHENYLAMINE	2	59	62
N-NITROSODIPROPYLAMINE	2	54	57
PENTACHLOROPHENOL	2	68	64
PHENANTHRENE	2	60	76
PHENOL	2	38	49
PYRENE	2	66	81
TRICHLOROBIPHENYL	2	60	75
D10-ACENAPHTHENE (B)	2	57	69
D10-FLUORENE (B)	2	57	71
D10-PYRENE (B)	2	62	76
D3-2,4-DICHLOROPHENOL (B)	2	57	63

(A) CORRECTED FOR THE AVERAGE AMOUNT FOUND IN THE BLANKS.
(B) RECOVERY STANDARD ADDED TO EACH FISH SAMPLE PRIOR TO EXTRACTION TO OTAIN AN INDICATION OF METHOD PERFORMANCE.
(C) THE COMPOUND WAS NOT DETECTED.

APPENDIX A -- DRAFT METHOD XXX -- DETERMINATION OF NEUTRAL AND ACIDIC PRIORITY POLLUTANTS IN FISH

This draft method is not intended to be an official analytical procedure. It represents the procedural steps followed in gathering the analytical data contained in the body of the report. The table and figures referred to in the text of the appendix may be found in EPA Method 625.

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May 24, 1985

Mr. Thomas Pressley
Environmental Monitoring Support Laboratory
U.S. Environmental Protection Agency
26 West St. Clair Street
Cincinnati, Ohio 45268

Dear Tom:

Enclosed is our draft copy of "Method XXX -- Determination of Base/Neutrals and Acids in Fish". If you have any questions please call me at (614) 424-4149.

Sincerely,

Tina

Tina M. Engel
Research Scientist
Analytical Chemistry Section

TME:lj

Enclosure

Method XXX -- Determination of Base/Neutrals and Acids in Fish

1. Scope and Application

1.1. This method covers the determination of a number of organic compounds that are partitioned into an organic solvent and are amenable to gas chromatography. The parameters listed in Table 1 may be qualitatively and quantitatively determined in fish tissue using this method.

1.2. The method may be extended to include the parameters listed in Table 2. Benzidine can be subject to oxidative losses during solvent concentration. Under alkaline conditions, alpha-BHC, delta-BHC, endosulfan I and II, and endrin are subject to decomposition. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition. N-Nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described. N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine.

1.3. This is a gas chromatographic/mass spectrometry (GC/MS) method applicable to the determination of the compounds listed in Tables 1 and 2 in fish tissue.

1.4. The method detection limit (MDL, defined in Section 16.1) for each parameter is listed in Table 3. The MDL for a specific fish tissue may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5. Any modification to this method, beyond those expressly permitted, shall be considered as a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5. Depending upon the nature of the modification and the extent of intended use, the applicant may be required to demonstrate that the modifications will produce equivalent results when applied to fish.

1.6. This method is restricted to use by or under the supervision of analysts experienced in the use of a gas chromatograph/mass spectrometer and in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1. A 25-gram sample of fish tissue is serially extracted by homogenization in acetonitrile. The acetonitrile extracts are combined with salted, buffered (pH 4) water in a bottle and partitioned with 50 percent methyl t-butyl ether (MTBE) in petroleum ether on a tumbler to remove fish lipid material from the extract. The organic extract is dried and concentrated. Gel permeation chromatography (GPC) is used as

a second cleanup procedure to remove lipid material from the extract. The extract is reconcentrated and analyzed by GC/MS. Qualitative identification of the parameters in the extract is performed using the compound retention time and mass spectrum. Quantitative analysis is performed using the internal standard technique with a single characteristic ion.

3. Interferences

3.1. Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the total ion current profiles. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.1.3.

3.1.1. Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by detergent washing with hot water, and rinses with tap water and distilled water. The glassware should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 min. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminate PCB interference. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2. The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2. Matrix interferences may be caused by contaminants that are co-extracted from the sample. Specifically, large amounts of lipid material are normally co-extracted from the fish tissue. The acetonitrile partitioning and GPC cleanup procedures outlined in Sections 10 and 11 are successful in removing most lipid material from the sample extracts. Unique situations may require additional cleanup procedures to achieve the MDLs listed in Table 3.

3.3. In samples that contain an inordinate number of interferences, the use of chemical ionization (CI) mass spectrometry may make identification easier. Table 3 gives characteristic CI ions for most of the compounds covered by this method. The use of CI mass spectrometry to support electron ionization (EI) mass spectrometry is encouraged but not required.

4. Safety

4.1. The toxicity or carcinogenicity of each reagent used in this method have not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference sheet should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identifed for the information of the analyst.

4.2. The following parameters covered by this method have been tentatively classified as known or suspected human or mammalian carcinogens: benzo(a)anthracene, benzidine, 3,3'-dichlorobenzidine, benzo(a)pyrene, alpha-BHC, beta-BHC, delta-BHC, gamma-BHC, dibenzo(a,h)anthracene, N-nitrosodimethylamine, 4,4'-DDT, and polychlorinated biphenyls (PCBs). Primary standards of these toxic compounds should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles / high concentrations of these toxic compounds.

5. Apparatus and Materials

5.1. Sample bottle -- 1-pint or appropriate size bottle, fitted with a screw cap lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. The bottle and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.2. Glassware (All specifications are suggested. Catalog numbers are included for illustration only.):

5.2.1. Centrifuge bottle -- 200-mL, equipped with Teflon-lined screw cap (Corning 1261 or equivalent).

5.2.2. Tumbler bottle -- 2500-mL, equipped with Teflon-lined screw cap (Wheaton Roller Culture Vessel 348524 or equivalent).

5.2.3. Flask, Erlenmeyer -- 500-mL.

5.2.4. Concentrator tube, Kuderna-Danish -- 25-mL, graduated (Kontes K-470050-2525 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5. Evaporative flask, Kuderna-Danish -- 500-mL (Kontes K-57001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6. Snyder column, Kuderna-Danish -- Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7. Snyder column, Kuderna-Danish -- Two-ball micro (Kontes K-569001-0219 or equivalent).

5.3. Boiling chips -- Carborundum, approximately 12 mesh (Hengar # 113-B or equivalent). Heat to 400° C for 30 minutes.

5.4. Water bath -- Heated, capable of temperature control (±1°C). The bath should be used in a hood.

5.5. Balance -- Analytical, capable of accurately weighing 0.0001 grams.

5.6. Homogenizer -- Capable of finely grinding fibrous tissue suspended in approximately 75 to 100 mL of solvent (Brinkmann PT 10-35 motor with PT 10 ST generator or equivalent).

5.7. GPC system:

5.7.1. Column -- 600 mm x 25 mm I.D. upward flow packed with 70 grams of BioBeads S-X3 resin. Resin should be swelled overnight in 50/50 methylene chloride/hexane prior to packing in the column.

5.7.2. Autosampler/Pump/Fraction Collector -- Pump capable of delivering 5 mL/min solvent flow through BioBeads S-X3 column. If desired, autosampler/fraction collector capable of delivering 5 mL of sample to the column and collecting predetermined column eluant fractions (ABC Model 1002A or equivalent).

5.7.3. UV detector -- Capable of handling 5-mL/min solvent flow without significant pressure build-up.

5.7.4. Syringe -- 10-mL, graduated, Leur-lock.

5.7.5. Filters -- 0.5 um, disposable, solvent resistant (Millipore SL SR025NS, or equivalent).

5.8. GC/MS system:

5.8.1. Gas Chromatograph -- An analytical system complete with a temperature programmable gas chromatograph and all required accessories including syringes, analytical columns, and gases. The injection port must be designed for splitless injection.

5.8.2. Column -- 30 m x 0.25 mm I.D. SPB-5, or equivalent. This column was used to develop the method performance statements in Section 16. Guidelines for the use of alternate columns are provided in Section 13.1.

5.8.3. Mass spectrometer -- Capable of scanning from 35 to 450 amu every 0.7 seconds or less, utilizing a 70 V (nominal) electron energy in the electron impact ionization mode, and producing a mass spectrum which meets all the criteria in Table 7 when 50 ng of decafluorotriphenylphosphine (DFTPP; bis(perfluorophenyl) phenylphosphine) is injected through the GC inlet.

5.8.4. GC/MS interface -- Any GC to MS interface that achieves all acceptable performance criteria (Section 12) may be used. It is recommended that the fused silica column described in Section 5.6.3 be vented directly into the mass spectrometer, avoiding the use of a separate interface.

5.8.5. Data system -- A computer system must be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer must have software that allows searching any GC/MS data file for specific ion and plotting such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundance in any EICP between specified time or scan number limits.

6. Reagents

6.1. Distilled Water.

6.2. Potassium phosphate, monobasic -- (ACS) Crystal.

6.3. Petroleum ether, acetonitrile, MTBE, methylene chloride, and hexane -- Distilled-in-glass quality or equivalent.

6.4. Sodium sulfate -- (ACS) Granular, anhydrous. Purify by heating at 400°C for 5 hours in a shallow tray.

6.5. Stock standard solutions (1.00 ug/uL) -- Standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.5.1. Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in MTBE or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or

greater, the weight may be used without correction to calculate the concentration of the stock standards. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.5.2. Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.5.3. Stock standard solutions must be replaced after six months or sooner if comparison with quality control check samples indicate a problem.

6.5.4. Recovery standard spiking solution -- Prepare a recovery standard spiking solution containing each of the recovery compounds listed in Table 8 at a concentration of 100 ug/mL in MTBE. Addition of 250 uL of this solution to 25 grams of sample is equivalent to a concentration of 1 ug/g of each recovery standard. Store the spiking solution at 4°C in a Teflon-sealed glass container. This solution should be checked frequently for stability. The solution must be replaced after six months or sooner if comparison with quality control check standards indicates a problem.

6.5.5. Internal standard/retention time standard/
column performance standard spiking solution -- Prepare a solution containing each of the internal, retention time, and column performance compounds listed in Table 6 at a concentration of 1000 ug/mL in MTBE. Addition of 20 uL of this solution to 1 mL of sample extracts yields a sample extract containing 20 ug/mL of each compound.

6.5.6. DFTPP standard -- Prepare a 25 ug/mL solution of DFTPP in acetone.

6.5.7. Quality control check sample concentrate -- See Section 8.2.1.

6.5.8 GPC calibration standard -- Prepare a solution containing 500 mg corn oil, 70 mg dioctylphenyl phosphonate, 50 mg 2,6-dibromophenol, and 6 mg sulfur in 100 mL of 50/50 methylene chloride/hexane.

7. Calibration

7.1. GPC calibration

7.1.1. Establish GPC operating parameters equivalent to those indicated in Figure 1.

7.1.2 Inject 5 mL of the GPC calibration standard (Section 6.5.8) onto the GPC column and monitor elution of standard components with a UVD. An example of a GPC-UVD chromatogram obtained from the analysis of the GPC calibration standard is shown in Figure 1.

7.1.3 Determine GPC fraction to be collected as shown in Figure 1.

7.2. GC/MS calibration:

7.2.1. Establish gas chromatographic operating parameters equivalent to those indicated in Figure 2.

7.2.2. Internal standard calibration procedure -- Quantification of identified compounds is done by the internal standard calibration. The recommended internal standard is given in Table 6. The analyst must demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Use the base peak ion as the primary ion for the quantification of the internal standard. If interferences are noted, use one of the next two most intense masses for quantification.

7.2.2.1. Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding appropriate volumes of one or more stock standards to a volumetric flask. To each calibration standard or standard mixture, add a known constant amount of the internal standard, retention time standard, and column performance standard spiking solution, and dilute to volume with methylene chloride. One of the calibration standards should be at a concentration near, but above the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC/MS system.

7.2.2.2. Using injections of 1 to 2 uL, analyze each calibration standard according to Section 13 and tabulate the area of the primary characteristic ion (Table 3) against concentration of each compound and internal standard. Calculate response factors (RF) for each compound using Equation 1.

Equation 1.

$$RF = \frac{[Ac][Cis]}{[Ais][Cc]}$$

where:

Ac = Area of the characteristic ion for the parameter to be measured.

Ais = Area of the characteristic ion for the internal standard.

Cis = Concentration of the internal standard (ug/mL).

Cc = Concentration of the parameter to be measured (ug/mL).

If the RF value over the working range is a constant (<35% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, Ac/Ais vs. RF.

7.3. The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±20%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

8. Quality Control

8.1. Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and an ongoing analysis of spiked samples to evaluate and document data quality. The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method. when results of sample spikes indicate atypical method performance, a quality control check standard must be analyzed to confirm that the measurements were performed in an incontrol mode of operation.

8.1.1. The analyst must make an initial, one-time demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2. In recognition of advances that are occurring in chromatography, the analyst is permitted certain options (detailed in Sections 10.6 and 13.1) to improve the separations or lower the cost of measurements. Each time such a modification is made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3. Before processing any samples, the analyst must analyze a reagent blank to demonstrate that interferences from the analyt-

ical system and glassware are under control. Each time a set of samples is extracted or reagents are changed, a reagent blank must be processed as a safeguard against laboratory contamination.

8.1.4. The laboratory must, on an ongoing basis, spike and analyze a minimum of 5% of all samples to monitor and evaluate laboratory data quality. This procedure is described in Section 8.3.

8.1.5. The laboratory must, on an ongoing basis, demonstrate through the analyses of quality control check standards that the operation of the measurement system is in control. This procedure is described in Section 8.4. The frequency of the check standard analyses is equivalent to 5% of all samples analyzed but may be reduced if spike recoveries from samples (Section 8.3) meet all specified quality control criteria.

8.1.6. The laboratory must maintain performance records to document the quality of data that is generated. This procedure is described in Section 8.5.

8.2. To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1. A quality control (QC) check sample concentrate is required containing each parameter of interest at a concentration of 100 ug/mL in methylene chloride. Multiple solutions may be required. PCBs and multicomponent pesticides may be omitted from this test. The QC check sample concentrate must be obtained from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory in Cincinnati, Ohio, if available. If not available from the source, the QC check sample concentrate must be obtained from another external source. If not available from either source above, the QC check sample concentrate must be prepared in the laboratory using stock standards prepared independently from those used for calibration.

8.2.2. Using a pipet, prepare QC check samples at a concentration of 1 ug/g by adding 250 uL of QC check sample concentrate to each of four 25-gram aliquots of control fish tissue.

8.2.3. Analyze the well-mixed QC check samples according to the method beginning in Section 10.

8.2.4. Calculate the average recovery ($\bar{X}$) in ug/g, and the standard deviation of the recovery (s) in ug/g for each parameter using the four results.

8.2.5. For each parameter compare s and $\bar{X}$ with the corresponding acceptance criteria for precision and accuracy, respectively, found in Table 4. If s and $\bar{X}$ for all parameters of interest meet

the acceptance criteria, the system performance is acceptable and analysis of actual samples can begin. If any individual s exceeds the precision limit or any individual $\bar{X}$ falls outside the range for accuracy, the system performance is unacceptable for that parameter.

Note. -- The large number of parameters in Table 4 present a substantial probability that one or more will fail at least one of the acceptance criteria when all parameters are analyzed.

8.2.6. When one or more of the parameters tested fail at least one of the acceptance criteria, the analyst must proceed according to Section 8.2.6.1 or 8.2.6.2.

8.2.6.1. Locate and correct the source of the problem and repeat the test for all parameters of interest beginning with Section 8.2.2.

8.2.6.2. Beginning with Section 8.2.2, repeat the test only for those parameters that failed to meet the criteria. Repeated failure, however, will confirm a general problem with the measurement system. If this occurs, locate and correct the source of the problem and repeat the test for all compounds of interest beginning the Section 8.2.2.

8.3. The laboratory must, on an ongoing basis, spike at least 5% of the samples from each sample site being monitored to assess accuracy. For laboratories analyzing 1 to 20 samples per month, at least one spiked sample per month is required.

8.3.1. The concentration of the spike in the sample should be determined as follows:

8.3.1.1. If, as in compliance monitoring, the concentration of a specific parameter in the sample is being checked against a regulatory concentration limit, the spike should be at that limit or 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.2. If the concentration of a specific parameter in the sample is not being checked against a limit specific to that parameter, the spike should be at 1 ug/g or 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration would be larger.

8.3.1.3. If it is impractical to determine background levels before spiking (e.g., maximum holding times would be exceeded), the spike concentration should be (1) the regulatory concentration limit, if any; or, if none (2)

the larger of either 5 times higher than the expected background concentration or 1 ug/g.

8.3.2. Analyze one sample aliquot to determine the background concentration (B) of each parameter. If necessary, prepare a new QC check sample concentrate (Section 8.2.1) appropriate for the background concentrations in the sample. Spike a second sample aliquot with 250 uL of the QC check sample concentrate and analyze it to determine the concentration after spiking (A) of each parameter. Calculate each percent recovery (P) as 100(A-B)%/T, where T is the known true value of the spike.

8.3.3. Compare the percent recovery (P) for each parameter with the corresponding QC acceptance criteria found in Table 4. These acceptance criteria were calculated to include an allowance for error in measurement of both the background and spike concentrations, assuming a spike to background ratio of 5:1. If spiking was performed at a concentration lower than 1 ug/g, the analyst must use either the QC acceptance criteria in Table 4, or optional QC acceptance criteria calculated for the specific spike concentration. To calculate optional acceptance criteria for the recovery of a parameter: (1) calculate accuracy (X') using the equation in Table 5, substituting the spike concentration (T) for C; (2) calculate overall precision (S') using the equation in Table 5, substituting X' for X; (3) calculate the range for recovery at the spike concentration as (100 X'/T)$\pm$2.44(100 S'/T)%.

8.3.4. If any individual P falls outside the designated range for recovery, that parameter has failed the acceptance criteria. A check standard containing each parameter that failed the criteria must be analyzed as described in Section 8.4.

8.4. If any individual P fails the acceptance criteria for recovery in Section 8.3, a QC check standard containing each parameter that failed must be prepared and analyzed.

Note. -- The frequency for the required analysis of a QC check standard will depend upon the number of parameters being simultaneously tested, the complexity of the sample matrix, and the performance of the laboratory. If the entire list of single-component parameters in Table 4 must be measured in the sample in Section 8.3, the probability that the analysis of a QC check standard will be required is high. In this case the QC check standard should be routinely analyzed with the spike sample.

8.4.1. Prepare the QC check standard by adding 250 uL of QC check sample concentrate (Sections 8.2.1 or 8.3.2) to 25 grams of control fish. The QC check standard needs only to contain the parameters that failed criteria in the test in Section 8.3.

8.4.2. Analyze the QC check standard to determine the concentration measured (A) of each parameter. Calculate each percent recovery (Ps) as 100(A/T)%, where T is the true value of the standard concentration.

8.4.3. Compare the percent recovery (P) for each parameter with the corresponding QC acceptance criteria found in Table 6. Only parameters that failed the test in Section 8.3 need to be compared with these criteria. If the recovery of any such parameter falls outside the designated range, the laboratory performance for that parameter is judged to be out of control and the problem must be immediately identified and corrected. The analytical result for that parameter in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

8.5. As part of the QC program for the laboratory, method accuracy for fish samples must be assessed and records must be maintained. After the analysis of five spiked fish samples as in Section 8.3, calculate the average percent recovery ($\bar{P}$) and the standard deviation of the percent recovery (sp). Express the accuracy assessment as a percent interval from P-2sp to P+2sp. If P=90% and sp=10%, for example, the accuracy interval is expressed as 70-110%. Update the accuracy assessment for each parameter on a regular basis (e.g. after each five to ten new accuracy measurements).

8.6. As a quality control check, the laboratory must spike all samples with the recovery standard spiking solution as described in Section 10.2, and calculate the percent recovery of each recovery compound.

8.7. It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to assess the precision of the environmental measurements. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1. Samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle can not be prerinsed with sample before collection.

9.2. All samples must be frozen from the time of collection until extraction.

10. Extraction and Acetonitrile Partitioning

10.1. Place 25 grams of fish tissue and 75 mL of acetonitrile into a 200-mL centrifuge bottle.

10.2. Homogenize sample for approximately 2 minutes using Polytron with Variac setting of 6 until tissue is finely dispersed in the acetonitrile.

10.3. Cap centrifuge bottle and centrifuge at 40,000 rpm for 20 minutes.

10.4. Pour supernatant into a 2500-mL tumbler bottle containing 1500 mL 2% sodium sulfate/1.5% monobasic potassium sulfate and 450 mL 50/50 petroleum ether/MTBE.

10.5. Add a second 75-mL volume of acetonitrile to the centrifuge bottle and repeat the homogenization procedure, combining the extracts in the tumbler bottle. Perform a third homogenization in the same manner.

10.6. Cap the tumbler bottle and tumble end-over-end at 30 turns/min for 1 hour. Allow contents of tumbler bottle to settle for 30 minutes.

10.7. Remove bulk of organic phase (top layer) to a 500-mL Erlenmeyer flask containing 10 grams of anhydrous sodium sulfate. Swirl contents of flask. Allow contents of flask to settle for 10 minutes.

10.8. Remove 360 mL of extract, representing 20 grams of fish tissue, to a 25-mL Kuderna-Danish (K-D) tube equipped with a 500-mL evaporative flask. Add 2 boiling chips to the K-D tube and attach a 3-ball Snyder column to the flask. Prewet the Snyder column with 1-2 mL of MTBE and place in a hot water bath (80-85°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of MTBE.

10.9. Add another one or two clean boiling chips to the concentrator tube and attach a two-ball micro-Snyder column. Prewet the Snyder column by adding about 0.5 mL of MTBE to the top. Replace the K-D apparatus in the hot water bath so that the concentrator tube is partially immersed in hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. When the apparent volume of liquid reaches about 1.0 mL, remove the K-D appartus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and

adjust the final volume to 10 mL with 50/50 methylene chloride/hexane. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored more than two days, they should be transferred to Teflonsealed screw-cap vials.

11. GPC Cleanup

11.1. Inject 5 mL of fish extract (Section 10.8) onto the GPC column and elute column with 50/50 methylene chloride/hexane at a flow rate of 5 mL/min. Collect fraction containing priority pollutant compounds as determined in Section 7.1.

11.2. Concentrate fraction to an apparent volume of 0.5 mL using K-D techniques as described in Sections 10.8 and 10.9. A higher bath temperature, 90-95°C, will be required. Dilute to a final volume of 1 mL with methylene chloride.

12. Daily GC/MS Performance Tests

12.1. At the beginning of each day that analyses are to be performed, the GC/MS system must be checked to see if acceptable performance criteria are achieved for DFTPP. In addition, the peak areas of the internal standard and column performance standards present in every sample and standard must be monitored on a run-to-run basis to see if acceptable performance criteria are maintained for the GC/MS system.

12.2. These performance tests require the following instrumental parameters:

Electron Energy:	70 V (nominal)
Mass Range:	35 to 450 amu
Scan Time:	To give at least 5 scans per peak but not to exceed 7 seconds per scan

12.3. DFTPP performance test -- At the beginning of each day, inject 2 uL (50 ng) of DFTPP standard solution. Obtain a background-corrected mass spectra of DFTPP and confirm that all the key m/z criteria in Table 7 are achieved. If all the criteria are not achieved, the analyst must retune the mass spectrometer and repeat the test until all criteria are achieved. The performance criteria must be achieved before any samples, blanks, or standards are analyzed.

12.4. Column performance standards/internal standard peak area monitoring -- Peak areas of the m/z of the column performance standards must be monitored for each sample analyzed. Variations of more than 50% from historical values signal the need for GC/MS system evaluation to determine and correct the source of the variation.

13. Gas Chromatography/Mass Spectrometry

13.1. The recommended gas chromatographic operating conditions for analyses of fish extract samples are summarized in Figure 2. The retention times and MDL that can be achieved under the recommended operating conditions are summarized in Table 3. Examples of separations achieved under these conditions are shown in Figure 2. Other capillary columns or packed chromatographic conditions may be used if the requirements of Section 8.2 are met.

13.2. After conducting the GC/MS performance tests in Section 12, calibrate the system daily as described in Section 7.

13.3. The internal standard and column performance standards must be added to the sample extract and mixed thoroughly immediately before injection into the instrument. This procedure minimizes losses due to adsorption, chemical reaction or evaporation.

13.4. Inject 1 to 2 uL of the sample extract or standard into the GC/MS system. Record the volume injected to the nearest 0.05 uL.

13.5. If the response for any m/z exceeds the working range of the GC/MS system, dilute the extract and reanalyze.

13.6. Perform all qualitative and quantitative measurements as described in Sections 14 and 15. When the extracts are not being used for analyses, store them refrigerated at 4° C, protected from light in screw-cap vials, equipped with unpierced Teflon-lined septa.

14. Qualitative Identification

14.1. Obtain EICPs for the primary m/z listed in Table 3. See Section 7.3 for masses to be used with internal and recovery standards. The following criteria must be met to make a qualitative identification:

14.1.1. The retention time must fall within $\pm$30 seconds of the retention time of the authentic compounds.

14.1.2. The entire mass spectra of the tentatively identified peak should be confirmed by an experienced MS operator.

14.2. Structural isomers that have very similar mass spectra and less than 30 seconds difference in retention time, can be explicitly identified only if the resolution between authentic isomers in a standard mix is acceptable. Acceptable resolution is achieved if the baseline to valley height between the isomers is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identifed as isomeric pairs.

15. Calculations

15.1. When a parameter has been identified, the quantitation of that parameter will be based on the integrated abundance from the EICP of the primary characteristic m/z in Table 3. Use the base peak m/z for internal and recovery standards. If the sample produces an interference for the primary m/z, use a secondary characteristic m/z to quantitate.

Calculate the concentration in the sample using the response factor (RF) determined in Secion 7.2.2 and
Equation 3.

$$\text{Concentration (ug/g)} = \frac{[Ac][Ic]}{[Ais][RF][Vc]}$$

where:

Ac = Area of the characteristic m/z for the parameter or recovery standard to be measured.
Ais = Area of the characteristic m/z for the internal standard.
Is = Amount of internal standard added to each extract (ug).
Vs = Weight of fish extracted (g).

15.2. Report results in ug/g without correction for recovery data. All QC data obtained should be reported with the sample results.

16. Method Performance

16.1. The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in Table 3 were obtained using fish. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

16.2. This method was tested by a single laboratory using two fish tissue matrices.